[COMMITTEE PRINT]

THE ENVIRONMENTAL IMPLICATIONS OF GENETIC ENGINEERING

STAFF REPORT

PREPARED BY THE

SUBCOMMITTEE ON
INVESTIGATIONS AND OVERSIGHT

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LETTER OF TRANSMITTAL

House of Representatives,
Committee on Science and Technology,
Washington, D.C., February 1, 1984.

Hon. Don Fuqua,
Chairman, Committee on Science and Technology,
U.S. House of Representatives.

Dear Mr. Chairman: I am pleased to transmit a report on the Environmental Implications of Genetic Engineering. The report is based on joint hearings held on June 22, 1983, by the Subcommittee on Investigations and Oversight, which I chair, together with the Subcommittee on Science, Research and Technology and an extensive review by the Investigations and Oversight Subcommittee staff. The report presents a summary of the staff review together with several recommendations.

Generally, the conclusions are:

(1) The potential environmental risks associated with the deliberate release of genetically engineered organisms are best described as "low probability of high consequence risks"; that is, while there is only a small possibility of occurrence, the damage that could occur is great.

(2) Predicting the specific type, magnitude or probability of environmental effects associated with deliberate release will be extremely difficult at the present time.

(3) The current regulatory framework does not guarantee that adequate consideration will be given to potential environmental effects of a deliberate release.

The report acknowledges vast potential benefits to be derived from biotechnology and recognizes the need to provide a clear and timely path for appropriate public review and agency decisionmaking on the products of biotechnology. To facilitate this goal, the report recommends an enhancement of EPA's expertise and efforts in biotechnology and the establishment of an interagency task force to review proposals, establish a risk assessment program, develop guidelines for environmental releases, and develop a public awareness capability. The panel shall also develop uniform guidelines for use in evaluating deliberate release proposals and consider how to adequately evaluate all releases.

The report also recommends that the NIH RAC review NIH-sponsored research and refer industrial requests to the appropriate agency and that both the NIH and USDA recombinant advisory committees should include persons specifically trained in the environmental sciences and ecology. Finally, the staff recommends that the General Accounting Office review and evaluate the authority of USDA to oversee environmental releases of genetically engineered products.

I would also like to extend my appreciation to Dr. Morris Levin, a LEGIS Fellow currently on assignment to the Investigations and Oversight Subcommittee, for his assistance in preparing this report.

Sincerely,

Albert Gore, Jr.,
Chairman, Subcommittee on
Investigations and Oversight.

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I. INTRODUCTION

On June 22, 1983, the Subcommittee on Investigations and Oversight and the Subcommittee on Science, Research and Technology held a joint hearing to examine the implications of deliberately introducing genetically engineered organisms into the environment. The purpose of the hearing was to consider the risks and benefits associated with the deliberate release of such organisms, as well as the adequacy of the existing federal regulatory framework to evaluate the potential environmental dangers presented by such releases. Impetus for the hearing came from concern that environmental implications may not be considered in sufficient depth and detail before the deliberate release of genetically engineered organisms on a large scale is authorized.

Attention has been increasingly focused on the environmental questions raised by deliberate releases because of the speed with which the biotechnology industry has developed in recent years. Biotechnology companies are rapidly approaching the point at which they will begin the widespread production of genetically engineered organisms for commercial purposes. These new organisms will range from genetically modified plants for enhancing the world food supply to genetically engineered microbes for hazardous waste clean-up. While these new life forms unquestionably offer many significant benefits to our society and our world, it is presently unclear what effect these new organisms will have on the environment into which they will be introduced.

It is recognized that the introduction of any novel organism could result in adverse environmental or public health impacts. The Subcommittees' inquiry, however, has been limited to questions related to genetically engineered organisms.

This report examines the potential benefits and risks presented by such deliberate releases and evaluates current scientific ability to assess the specific risks posed by them. The development of the capability to predict accurately the effects of an organism on the environment is essential if new genetically engineered products are to achieve widespread feasibility and the benefits of biotechnology are to be realized. The ability to predict is important not only to avoid detrimental impacts but also to determine when no such dangers are present.

Two past instances of exotic introduction can be used to illustrate the value of accurate predictions of the impact of a new organism on an ecosystem. One involved the introduction of striped bass into a Guatemalan lake; the other involved the introduction of Nile perch into a lake in Uganda. In the first case, a prediction of disaster was correct: the bass destroyed the native fishery. In the second case, however, a similar prediction of catastrophe was totally in error: the perch flourished and provided needed food and economic benefits to the people in the region. If the advice of the biologists had been followed in the first case, the environment -- as well as the economy -- of the affected area would not have been damaged. If the biologists' advice had been followed in the second case, many great benefits would have been missed.

These two examples, as well as such instances as Kudzu, Gypsy Moth and Dutch Elm disease may not be directly analagous to deliberate release of genetically engineered organisms but they do underscore the problems that can arise from an inability to predict the effects that a new organism might have on an ecosystem. Assessing the risks presented by deliberate releases should not be simply a game of biological roulette. If it is, many benefits of this new technology will be lost, and disastrous environmental consequences could well be permitted to occur.

Although this report focuses specifically on the environmental implications of deliberate releases, the potential risks to public health created by such releases should not be discounted. Indeed, concern over the possible effects of releases on human health underlies the examination of this issue. However, that issue must be the focus of a separate inquiry.

This report addresses the deliberate release of all "genetically engineered" organisms and not just those created through recombinant DNA techniques. While much of the discussion in the report involves recombinant DNA technology -- the actually cutting and splicing of genes -- "genetic engineering", encompasses far more than that particular technology. It is intended to include as concerns about the potential environmental effects of genetically engineered organisms created by other techniques -- such as protoplast fusion (the fusing together of two unwalled cells), chemical mutation, and other procedures -- as about the possible impacts of organisms created by recombinant DNA technology described by the RAC in their charter.

A. BACKGROUND

The possibility of creating a genetically engineered organism derives from advances in the means of synthesizing, analyzing, transposing, and transporting deoxyribonucleic acid (DNA) in and between organisms. As recombinant DNA technology was being developed in the mid-1970s, its first applications were focused in the area of human health care. Because much of the work being done at that time involved the use of infectious organisms, a good deal of scientific and public concern existed that dangerous microbes could escape from the laboratory and endanger the public health and safety.

This led to the establishment of guidelines in 1976 by the National Institutes of Health (NIH) to govern the conduct of recombinant DNA research. These guidelines prescribe rules for the containment of microbes containing recombinant DNA in the laboratory and are intended to ensure that the benefits of recombinant DNA research are achieved without the risk of an accidental escape of an organism. The guidelines legally apply only to research funded by the NIH, but other agencies have applied them as well. Moreover, it is believed that private industry has, for the most part, complied with them voluntarily. The existence of these guidelines has resulted in safe laboratory conditions, no adverse effects, and a diminished concern over accidental escape of engineered microbes.

As the safety of recombinant DNA research itself has been demonstrated, emphasis on the commercial aspects of biotechnology has increased. Major achievements in the ability to cleave DNA at specific sites, along with the development of methods to rejoin the severed ends to produce a recombinant molecule (RDNA) and the discovery of effective techniques for introducing RDNA into other organisms, have given scientists the ability to alter many kinds of organisms rapidly and effectively. Spurred by the Supreme Court's decision in 1980[1] in Diamond v. Chakrabarty, which permitted the patenting of new life forms, the biotechnology industry has burgeoned over the last few years and has begun to make substantial progress toward commercially feasible genetically engineered products. Recent research accomplishments in the genetic modification of microorganisms, plants, and animals appear to have wide potential application in both agriculture and industry.[2] Potential new products include plants with greater disease resistance; bacteria that enhance the nitrogen-fixing capability of plants; microbes that detoxify hazardous wastes, clean up oil spills, or facilitate the recovery of minerals from the ground; and many, many others.

As the new technology has advanced, however, a new type of public concern has arisen. In contrast to the previous attention to the harm that could result from the accidental escape of a new genetically engineered organism, concern now exists over the possible effects from the deliberate release of one. Because they are designed to produce results in the field rather than in the laboratory, effective performance of these new products will necessitate the deliberate release of a genetically modified organism into the environment. Although no commercial biotechnology firm has yet advanced to the point where large-scale deliberate releases are imminent, that time is rapidly approaching.[3] Commercial applications are becoming increasingly feasible, and as more modified organisms become available, introductions into the environment will likely occur on a more widespread basis and on a far greater scale. Introduction of these organisms could have profound effects on the environment and the ecological balance that presently exists.

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[1] 447 U.S. 303 (1980).

[2] The Congressional Office of Technology Assessment (OTA) in 1981 documented the history, methods, and wide range of applications and effects that may be anticipated as biotechnology is developed by various industrial sectors. The report indicates that there are applications to health, food, energy, and environmental problems. The report also discusses the various methods by which genetically engineered organisms can be produced: transformation, transduction, and protoplast fusion, as well as in vitro rearrangement and reinsertion of genetic material. See Congressional Office of Technology Assessment, Impacts of Applied Genetics (1981) [hereinafter cited as OTA Report].

Specialized microorganisms, rather than modified plants or animals, will likely be the first products of biotechnology introduced broadly into the environment. This is because of the relative ease with which they can be genetically manipulated. Indeed, one product has already been patented,[4] and field tests on two other microbes have been approved.[5] Because microorganisms have the ability to mutate quickly in nature and to multiply extremely rapidly when conditions are favorable, the deliberate introduction of microorganisms with novel genotypes raises particularly significant questions of control and containment.[6]

Although the widespread introduction of modified plants and animals into the environment is farther in the future, there are nonetheless major environmental issues raised by current research activities in these areas as well. Field tests of new genetically engineered plants have already been approved by NIH,[7] and laboratory experiments resulting in genetically modified organisms with environmental implications, such as the "super mice", have been conducted.[8]

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[3] See Response of Dr. Alexander to supplemental written questions in Appendix B to the hearing transcript.

[4] The product designed for deliberate release that has been patented is the microbe for use in cleaning up oil spills designed by Dr. Chakrabarty. This microbe was the subject of the Supreme Court's decision in Diamond v. Chakrabarty, 447 U.S. 303 (1980).

[5] 48 Fed. Reg. 24549 (June 1, 1983) (Part II).

[6] See, e.g., the response of Dr. Alexander to supplemental written questions contained in Appendix B to the hearing transcript; Hardy testimony p. 82.

[7] Federal Register, August 7, 1981 (p.40331); April 15,1983 (p.16459).

The novelty of genetically engineered organisms and the lack of experience with them in the environment make it difficult to predict the impact their introduction might have on an ecosystem. As the experiences with the introduction of other, more familiar organisms, such as the Gypsy Moth, reveal, however, serious problems can arise if adequate environmental protection does not exist at the time of the release.[9] In fact, because biotechnology is now producing countless genetically engineered organisms with genotypes that did not previously exist (except perhaps as isolated, individual organisms), the potential for ecological disruption may be greater for these "nonnatural" organisms than for any natural entity.

B. Issues

The issues addressed at the hearing were:

- What are the benefits and risks associated with the release of organisms with new genotypes into the environment?
- Is it possible to predict the impact of the introduction of a particular organism into a given environment?
- Is the existing regulatory framework adequate to assess the risks and benefits associated with the deliberate release of a genetically engineered organism and thereby permit the benefits of the technology to be realized while minimizing the risk of an adverse environmental impact?

C. Witnesses

The Subcommittees heard testimony from nine witnesses representing academia, industry, and government. The witnesses and the panels on which they appeared were:

Panel One: Environmental Effects

Dr. Martin Alexander, Cornell University. Dr. Alexander is an eminent microbial ecologist who serves on the faculty of the Department of Agronomy at Cornell. Dr. Alexander was formerly a member of EPA's Science Advisory Board and chaired an EPA study group on recombinant DNA policy.

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[8] See, e.g., Science, November 18, 1983, at p.809; Nature, December 16, 1982, at p.611.

[9] See the discussion in Part IV(A) of this report.

Dr. Frances Sharples, Oak Ridge National Laboratory (ORNL). Dr. Sharples is a terrestrial ecologist who now serves as an administrator at ORNL. She was formerly assigned to ORNL's Environmental Sciences Division. In October 1982, Dr. Sharples published a study for EPA on the effects of the introduction of organisms with novel genotypes into the environment. This report has also been published in the NIH's Recombinant DNA Technical Bulletin.

Mr. Geoffrey Karny, Office of Technology Assessment (OTA). Mr. Karny, a Senior Analyst with the OTA at the time of the hearing, is an attorney who has been heavily involved in evaluating the regulatory aspects of new products and processes resulting from developments in biotechnology. Mr. Karny participated in the 1981 OTA study, Impacts of Applied Genetics, and the 1983 OTA study, Biotechnology: Commercialization and International Competitiveness. He is currently a practicing attorney in Washington, D.C.

Panel Two: Research Activities

Dr. Ananda Chakrabarty, University of Illinois. Dr. Chakrabarty is a Professor of Microbiology at the University of Illinois, Chicago campus, and is a leading scientist in the field of genetic engineering. Dr. Chakrabarty's research on microbes for oil spill clean-ups was the subject of the United States Supreme Court's decision in 1980 in Diamond v. Chakrabarty, which allowed the patenting of life forms. In addition to his work on the oil microbes, Dr. Chakrabarty has been involved in research to develop microbes to detoxify hazardous wastes, such as dioxin.

Dr. Steven Lindow, University of California. Dr. Lindow is an Assistant Professor of Plant Pathology at the University of California, Berkeley. Recently, Dr. Lindow and his colleagues succeeded in deleting from certain bacteria the gene that promotes the formation of ice crystals on plants. As a result of Dr. Lindow's research, it may soon be possible to replace the natural bacteria on plants with genetically modified bacteria and thereby achieve protection against frost damage.

Dr. Ralph Hardy, DuPont Corp. Dr. Hardy is the Director of Life Sciences Research at DuPont. DuPont is one of the leading major companies involved in genetic engineering research in a variety of areas.

Panel Three: Government Oversight

Dr. Bernard Talbot, National Institutes of Health (NIH). Dr Talbot is the Deputy Director of the NIH's Institute of Allergy and Infectious Diseases. Dr. Talbot has responsibility within NIH for overseeing the activities of the Office of Recombinant DNA Activities.

Mr. Donald Clay, Environmental Protection Agency (EPA). Mr. Clay is the Acting Assistant Administrator for Pesticides and Toxic Substances for EPA. His office has principal responsibility at EPA for implementing the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA).

Dr. Edgar Kendrick, Department of Agriculture (USDA). Dr. Kendrick is the Acting Deputy Assistant Secretary for Science and Education.

II. SUMMARY OF FINDINGS

The findings are based on the testimony presented to the Subcommittees at the hearing, information obtained during the course of our investigation, and responses by the witnesses to supplemental written questions submitted to them by the Subcommittees.

A. Benefits and Risks

Issue:

What are the benefits and risks associated with the release of organisms with new genotypes into the environment?

Findings:

The testimony presented to the Subcommittees described both the benefits and risks associated with the introduction of organisms with new genotypes into the environment. The potential benefits include such desirable ends as the degradation of toxic/hazardous material, enhanced food production, enhanced energy production, and easier extraction of minerals with less environmental damage. The risks are less easy to define and depend upon the nature of the organism released. Overall, the risk presented by the deliberate release of a genetically engineered organism is that it may cause environmental changes that perturb the ecosystem it encounters and/or that the organism itself may have negative effects if it establishes itself outside of the specific environment for which it was intended. Although no detrimental effects of any genetically engineered organism on an ecosystem have been documented, severe negative and beneficial impacts from newly introduced naturally occurring organisms are well known. The potential environmental risks associated with the deliberate release of genetically engineered organisms or the translocation of any new organism into an ecosystem are best described as "low probability, high consequence risk"; that is, while there is only a small possibility that damage could occur, the damage that could occur is great.

B. Environmental Impact

Issue:

Is it possible to predict the impact of the introduction of a particular organism into a given environment?

Findings:

The testimony presented to the Subcommittees indicated that predicting the specific type, magnitude, or probability of environmental effects associated with the deliberate release of genetically engineered organisms will be extremely difficult, if not impossible, at the present time. This is principally the case because no historical and scientific data base exists concerning the behavioral characteristics of genetically engineered organisms in the environment and no standard ecological methodology for predicting the outcome of an exotic introduction current exists. In addition, as experiences with naturally occurring organisms have demonstrated, it is possible to make only an imprecise estimate, at best, of the effect that an organism may have on the environment. Nevertheless, the testimony indicated that it would be possible to devise procedures to produce generalized estimates of the probability of environmental damage by, and survival and growth of, a genetically engineered organism, although specific risk assessment may not be achievable.

C. Regulatory Framework

Issue:

Is the existing regulatory framework adequate to assess the risks and benefits associated with the deliberate release of a genetically engineered organism and thereby permit the benefits of the technology to be realized while minimizing the risk of an adverse environmental impact?

Findings:

Based on our investigation of the existing oversight mechanisms for biotechnology, it is clear that the current regulatory framework does not guarantee that adequate consideration will be given to the potential environmental effects of a deliberate release. No single agency or entity presently has both the expertise and authority to properly evaluate the environmental implications of releases from all sources. Although some federally funded researchers and commercial biotechnology firms presently request approval from the National Institutes of Health's (NIH) Recombinant DNA Advisory Committee (RAC) for deliberate releases, the RAC's ability to adequately evaluate the environmental hazards posed by deliberate releases is limited by both its expertise and its jurisdiction. The scope and content of the Environmental Protection Agency's (EPA) regulatory effort is, at present, unknown, and that agency's ability is limited by its minimal experience in genetic engineering matters and unresolved questions concerning jurisdiction under the Toxic Substances Control Act (TSCA). The Department of Agriculture (USDA) may have jurisdiction to oversee deliberate releases under several statutes, but USDA's role is unclear because of its limited experience and disinclination toward oversight in this area.

III. RECOMMENDATIONS

The following recommendations are based on the findings outlined above:

(1) The EPA should proceed with its stated intention to extend its authority to include all deliberately released organisms not specifically identified as part of the legal obligation of another agency. In view of EPA's stated conclusion that the Toxic Substances Control Act (TSCA) does provide it with authority to oversee deliberate releases and the fact that Congress intended TSCA to be "gap filling" legislation, no additional legislation or clarifying amendments are needed at this time. EPA should, however, establish formal communications and agreements with other agencies to ensure that gaps and redundancies in the regulatory structure do not occur. A major goal should be to permit research and commercialization to proceed with minimum interference while adequately addressing environmental and public health concerns.

(2) Until such time as EPA's regulations are promulgated, an interagency task force should be established to review all proposals for deliberate releases. EPA should take the initiative in organizing this panel. The panel should be comprised of representatives from EPA, USDA, NIH, and any other appropriate federal agency or entity directly involved from either the scientific or regulatory perspective. The panel should establish an environmentally oriented risk/benefit assessment program to evaluate current proposals for deliberate releases and to provide a data base for decisions on future releases. The panel should also develop a uniform set of quidelines to govern deliberate releases. The panel should, moreover, serve the function of educating the public about the potential risks and benefits associated with this aspect of biotechnology. Consideration should be given to making this panel a permanent oversight body even after EPA has promulgated regulations to ensure that the broadest possible expertise is brought to bear in overseeing the technology.

(3) No deliberate release should be permitted by EPA, NIH, USDA, or any other federal agency until the potential environmental effects of the particular release have been considered by the interagency review panel. The panel shall consider the effects of any environmental release, regardless of size or intent. Each agency should evaluate proposals for deliberate releases according to a uniform set of guidelines to be developed by the interagency task force. It is recognized that initially decisions may be made on the basis of incomplete data.

(4) The task force should consider the need for oversight of research scale releases and, if appropriate, develop guidelines

for reviewing proposals for such releases. The task force should prepare a report containing its conclusions on this matter within 90 days of its establishment. The report should be made available to the Subcommittee.

(5) The NIH should cease its practice of evaluating and approving proposals for deliberate releases from commercial biotechnology companies. The NIH should review proposals only from parties engaged in NIH-sponsored research, and refer requests from industry to the appropriate agency.

(6) The NIH and USDA should revise the membership of their respective Recombinant DNA Advisory Committees (RAC) to include individuals specifically trained in ecology and the environmental sciences.

(7) The General Accounting Office should review the activities of USDA in overseeing biotechnology and evaluate the agency's authority to regulate deliberate releases under all relevant statutes, regulations, and executive orders.

IV. DISCUSSION OF ISSUES, FINDINGS, AND RECOMMENDATIONS

A. Benefits and Risks

Issue:

What are the benefits and risks associated with the release of organisms with new genotypes into the environment?

Findings:

The testimony presented to the Subcommittees described both the benefits and risks associated with the introduction of organisms with new genotypes into the environment. The potential benefits include such desirable ends as the degradation of toxic/hazardous material, enhanced food production, enhanced energy production, and easier extraction of minerals with less environmental damage. The risks are less easy to define and depend upon the nature of the organism released. Overall, the risk presented by the deliberate release of a genetically engineered organism is that it may cause environmental changes that perturb the ecosystem it encounters and/or that the organism itself may have negative effects if it establishes itself outside of the specific environment for which it was intended. Although no detrimental effects of any genetically engineered organism on an ecosystem have been documented, severe negative and beneficial impacts from newly introduced naturally occurring organisms are well known. The potential environmental risks associated with the deliberate release of genetically engineered organisms or the translocation of any new organism into an ecosystem are best described as "low probability, high consequence risk"; that is, while there is only a small possibility that damage could occur, the damage that could occur is great.

Discussion:

Testimony before the Subcommittees strongly indicated that the benefits -- both direct and indirect -- associated with deliberate releases outweigh the known risks. The need to better understand the effects of the deliberate introduction of novel organisms into an ecosystem was stressed, however. It was clear that although the potential negative effects may be great, the probability of an effect is thought to be very low.[10]

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[10] The discussion in this section addresses the particular, direct risks and benefits associated with given deliberate releases. It is recognized that other ancillary benefits or risks to society may result from a deliberate release. Discussion of these questions is beyond the scope of this report.

One benefit discussed by the witnesses was the enhancement of the world food supply through genetic engineering techniques. According to Dr. Ralph Hardy, the food supply must increase at or above the rate of increase of the world population. Considering the shrinkage of arable land, the present rate of increase in crop production (three percent annually) will not provide the supply of inexpensive nutritious foodstuffs that will be required by the estimated 6 billion persons who will inhabit the Earth by the year 2000.[11]

Genetically engineered crops with improved nutritional qualities and increased herbicide and disease resistance will permit higher yields per acre and help avoid food shortages. Already, plants with altered storage proteins and increased herbicide resistance are relatively close to reality, and attempts to confer increased nitrogen-fixing capability (i.e., the ability to utilize nitrogen more directly) in plants are being made in several laboratories.[12]

Progress is also being made toward the development of disease-resistant plants and toward the selection of disease-free stocks for conventional plant breeding. Additionally, work is being conducted on the regulation and coordination of gene expression in order to design genes to produce chemicals that will turn other genes on and off at critical stages in a plant's growth. The thrust of this research is to effect beneficial changes in plant growth development and metabolism.[13]

In addition to these processes aimed at manipulation of plants' genetic structures, testimony was also presented describing the use of molecular biology techniques to produce altered microbes (Pseudomonas syringea and Erwinia herbicola) that can reduce frost damage in plants.[14] The use of these microbes could reduce crop loss by one to three billion dollars per year.[15]

Deliberate releases could also result in enhanced food production by increasing our pesticide utilization capability. The development of suitable degradative organisms and techniques for their application may permit the use of insecticides or pesticides that are currently banned because of long-term toxicity or dispersal problems.[16]

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[11] Hardy testimony, p. 78-79.
[12] Reseachers are attempting to both alter the plants directly and increase the ability of common soil microbes to serve as sources of nitrogen. While the development of altered plants is still in the future, microbes with enhanced nitrogen-fixing ability have already been created. Hardy testimony p.82.
[13] Hardy testimony p.80-81.
[14] Lindow testimony p.68-70.
[15] Lindow testimony p.67.

While new genetically engineered plants offer the possibility of tremendous benefits to society, there are also potential risks associated with the introduction of these new plants into the environment. As stronger strains of plants are developed and ways to enhance soil constituents are discovered, the danger exists that without proper safeguards, these new plants could transfer genetic material to other plants, perhaps resulting in increased, rather than decreased, crop susceptibility to disease.[17] Moreover, there could be alterations in the niches and pathogenicities of plant viruses and soil bacteria, or even the development of more vigorous and resistant weeds, with an increase in the need for herbicides. It is also possible that these plants may create problems if they expand from their designated areas into other niches.

Other benefits to society include enhancing the quality of the environment and the development of procedures for producing needed material and energy in a less environmentally damaging manner.

The potential use of genetically engineered organisms to treat soil or water contaminated with toxic chemicals was discussed by several witnesses. For example, new microbes are being created that will "eat" hazardous waste by breaking it down into nontoxic components. These organisms (Pseudomonas species) are capable of degrading pollutants ranging from TCDD (dioxin) to oil spills.[18] It was also pointed out that the presently available alternatives for waste disposal -- such as incineration,

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[16] Hardy testimony p.78-79.

[17] Through a process known as transduction (whereby foreign genes become inserted into a host organism) or through cross-pollination, it is possible that the modified genes of a genetically engineered plant could be transferred to surrounding plants. As Dr. Sharples pointed out, "Bacteria are known to be able to exchange plasmids between different species and even genera... . Thus, the introduction of an engineered organism into the environment may have not only the potential to cause the kind of ecological disruption that has been associated with some 'exotic' species, but also may have the potential to pass on 'novel' genes into wild organisms." Response of Dr. Sharples to supplemental written questions in Appendix (A) to the hearing transcript. The possibility that modified genes could be transmitted from one organism to another may depend on the process employed to make the original genetic modification. For example, Dr. Lindow pointed out that the techniques he has employed result in microbes that behave similarly to chemically induced mutant organisms. According to Dr. Lindow, the use of mutants

(Footnote continued)

burial, and isolation -- are much more expensive and less than satisfactory. Incineration has a high energy demand, and the long term outlook for burial and isolation is at best uncertain.

The benefits to be derived from these "bugs" are obvious, but there are also potential risks involved with their usage. If these microbes were to move beyond the waste and begin to break down other compounds in the environment, or if the break-down products themselves are toxic, serious damage could occur.

Genetically engineered organisms can also increase our nation's energy and mineral resources. Microbially enhanced Oil Recovery (EOR) is a procedure being explored by a number of oil producers whereby microbes are injected into wells to permit recovery of some of the 66% of the oil normally left in the ground. Microbes are also being designed to facilitate mining and mineral leaching. Moreover, research is also currently being conducted on the role that genetically engineered microbes can play in facilitating the conversion of biomass into energy.[19]

Again, while the benefits of such applications would be great, there are potential environmental risks. For example, microbes inserted into the ground for oil recovery, mining, or mineral leaching could also enhance the generation of sulfuric and other acids. An increase in the presence of these substances could cause serious acidification of our fresh water resources.

Mr. Clay succinctly summarized the potential dangers presented by deliberate releases as falling into three general categories:

(1) ecological disruption due to lack of natural enemies;

(2) infectivity, pathogenicity, or toxicity to nontarget organisms (plants, animals, humans); and

(3) exchange of genetic material with other organisms or disrupting ecosystems.

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[17] (continued)
constructed in vitro eliminates the possibility that any silent mutations could have future adverse effects on an ecosystem. In Dr. Lindow's experiment, the ice nucleation gene is removed from the bacteria that cover the plants. The resulting genetically modified microbe is theoretically more stable than those produced by other techniques since the entire gene is deleted from it. The deletion of the entire gene eliminates any possibility of reversion. Thus, the organism theoretically will produce only the result for which it has been designed. Lindow testimony p.70-71.

[18] Chakrabarty testimony p.62-64.

[19] See OTA Report, supra note 2, at 117-23.

"Because of the large amount of uncertainty and the potential magnitude associated with these risks," Mr. Clay asserted, "...it is appropriate to take a cautious approach in the release of genetically engineered microorganisms into the environment."[20]

Currently, the only entity actively reviewing proposals for deliberate releases, and thereby theoretically assessing the risks and benefits associated with such releases, is the Recombinant DNA Advisory Committee (RAC) at the National Institutes of Health.

To date three cases of deliberate release have been approved by the RAC.[21] The three are:

o Dr. Ronald Davis, Stanford University, to field test corn plants transformed with corn plant (Zea mays) DNA. Dr. Davis is attempting to improve the dietary value of corn by adding genes which would increase the storage protein in each kernel. The field experiment was patterned after existing protocols developed for field testing new plant species produced by cross fertilization. Although approved in August 1981, the field trials have not been conducted.

o Dr. John Sanford, Cornell University, to field test tomato and tobacco plants (Solaraceae family) transformed with bacterial and yeast DNA. The objective of the trials is to demonstrate that pollen would serve as a vector for the inserted DNA. It was estimated that thousands of plants would be involved in detecting transformants in the field. Approval for the tests was given in April 1983, but the trials have not been conducted.

o Drs. Nickolas Panapoulos and Steven Lindow, University of California, to field test syringae and herbicola from which the genes for producing ice crystal nuclei have been removed. The purpose of this experiment is to develop microbes that will reduce the susceptibility of crops to frost damage. Approval for the tests was given in June 1983, but the trials have not yet been conducted.[22]

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[20] Response of Mr. Clay to supplemental written questions in Appendix B to the hearing transcript.

[21] Federal Register August 7, 1981 (p. 40331); April 15, 1983 (p.16459); 48 Fed. Reg. 24549 (1983) (Part II).

[22] The field tests by Drs. Davis and Sanford were never conducted because the laboratory data did not indicate that the tests would be successful. In contrast, the testing of the microbes developed by Drs. Panapoulos and Lindow has been delayed at least in part because of a lawsuit filed recently that challenges the legitimacy of the NIH's procedures for approving deliberate releases. The suit also questions the safety of this particular experiment.

(Footnote continued)

In addition to these three proposals by government-sponsored researchers, two industrial proposals involving the deliberate release of genetically engineered organisms have been reviewed by the RAC. Neither proposal was given full approval, however.[23]

Because none of the field tests have actually been conducted, there is presently no evidence of the impact that genetically engineered organisms could have on the environment. This lack of data makes identification of specific risks impossible at this time. The need for additional data in order to determine the possible risks was emphasized by Drs. Sharples and Alexander, both of whom stressed the uncertainties associated with introducing novel organisms into established ecosystems.[24]

Despite the absence of data relating specifically to any negative environmental effects of genetically engineered organisms, most witnesses urged caution in reviewing proposals for deliberate releases.[25] Witnesses noted that past experiences with the introduction of non-genetically engineered, non-native organisms provide some indication of the problems that can arise when inadequate attention is paid to environmental considerations. For example, Gypsy Moths were imported from Europe in the late 1800s for research purposes and later accidentally released into the environment. Once released, these moths multiplied rapidly, and today, large numbers of them periodically defoliate millions of acres of trees across the country. Starlings were intentionally imported from England and released in this country in the 19th century. Now annually they cause millions of dollars in crop damage here. Microorganisms brought into this country

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[22] (continued)
Specifically, the suit alleges that the bacteria to be applied to plants by Drs. Panapoulos and Lindow might become detached from the plants and escape into the environment, with possibly disastrous ecological consequences. See Foundation on Economic Trends v. Heckler, No. 83-(2714) (D.D.C. Sept. 14, 1983). See also note 110 infra.

[23] The proposals were submitted by Cetus Madison Corporation and by Biotechnica International. Biotechnica proposed to treat alfalfa plants with a genetically engineered bacterium designed to enhance the nitrogen-fixing capability of the plants. This request was denied by the RAC, because there was insufficient information for any type of a decision. Cetus proposed to test a plant that was made more disease resistant by genetic engineering. The RAC neither approved nor denied this request; instead it requested further information from Cetus and referred the proposal to the RAC Plant Working Group.

[24] Testimony p.51.

[25] See e.g., response of Mr. Clay to supplemental written questions in Appendix B to the hearing transcript.

from abroad have destroyed large numbers of chestnut and elm trees. Kudzu and other plants have been introduced into the South and Southwest and have dominated other flora. Many other examples exist.[26]

Although no genetically engineered organism has yet been released into the environment and consequently no specific data on environmental effects in the field exists, some data is available on environmental effects determined in the laboratory. In 1977, two scientists in New Zealand, K.L. Giles and H.C.M. Whitehead, attempted to enhance the nitrogen fixing capability of a certain species of pine tree by genetically modifying a fungus that inhabited the trees. In seeking to modify this fungus, the scientists combined two normally nonpathogenic microorganisms. This combination theoretically should have produced a harmless fused organism capable of bringing about the desired result. In contrast, however, one strain of the newly created recombinant fungus was in fact pathogenic and one isolate killed tree seedlings to which it was applied.[27]

It must be noted that while the effect of the modified organism was clearly demonstrated on the seedlings, it is equally possible that the pathogenic nature of the new organism could have gone unnoticed for many years. There are disease-producing organisms, such as the chestnut blight, that affect only mature trees and have no effect on seedlings. Consequently, a disease of this type would not have been detected by the procedures used in this experiment.

This experiment is not cited to indicate that all genetically

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[26] An excellent discussion of these and other cases is contained in the report prepared by Dr. Sharples for EPA. See F. Sharples, Spread of Organisms with Novel Genotypes: Thoughts from an Ecological Perspective (October 1982) in Appendix A to the hearing transcript.

[27] See Giles and Whitehead, Reassociation of a Modified Mycorrhza with the Host Plant Roots (Pinus Radiata) and the Transfer of Acetylene Reduction Activity, 48 Plant and Soil 143-52 (1977; See also Giles and Whitehead, The Transfer of Nitrogen Fixing Ability to a Eucaryotic Cell, 14 Cytobios 49-61 (1975), Appendix A. Electron microscopy showed that the hyphae of the modified strains were found in the intercellular spaces of the plants, as was to be expected. The hyphae were also found within the cells of the root cortex, however. No such intercellular growth was found with the "Wild Root" or "Control" strains. The line between symbiosis and pathogenisis has long been recognized as being very fine, and in this particular case it appears that the relationship shifted away from being mutually beneficial. Since the procedure used involved transferring the entire genome into the host and selecting for a

(Footnote continued)

transformed organisms are -- or can be -- dangerous. As noted, many single gene alterations have no effect on the environmental or impact of an organism, and typically such changes result in mutations that are lethal only to the organism itself. However, the experiment illustrates, albeit on a very limited scale, the potential damage that could result from a deliberate release. The experiment is cited simply as a real example of what could occur and demonstrates why a thorough examination must be made of the potential environmental effects of an organism before large-scale field tests are undertaken.

B. Environmental Impact

Issue:

Is it possible to predict the impact of the introduction of a particular organism into a given environment?

Findings:

The testimony presented to the Subcommittees indicated that predicting the specific type, magnitude, or probability of environmental effects associated with the deliberate release of genetically engineered organisms will be extremely difficult, if not impossible, at the present time. This is principally the case because no historical and scientific data base exists concerning the behavioral characteristics of genetically engineered organisms in the environment, and no standard ecological methodology for predicting the outcome of an exotic introduction currently exists. In addition, as experiences with naturally occurring organisms have demonstrated, it is possible to make only an imprecise estimate, at best, of the effect that an organism may have on the environment. Nevertheless, the testimony indicated that it would be possible to devise procedures to produce generalized estimates of the probability of environmental damage by, and survival and growth of, a genetically engineered organism, although specific risk assessment may not be achievable.

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[27] (continued)
particular characteristic (in this case Acetylene reduction as a surrogate for Nitrogen Fixation) there was no provision for identifying other characteristics which may have been transferred simultaneously. It should be noted that because this procedure employed techniques not covered by the RAC charter and was used for research, rather than commercial, purposes, the experiment would not have come under the jurisdiction of the RAC or any other agency had it been conducted in the United States. All cultures, soil, and plants were sterilized upon termination of the experiment.

Discussion:

The testimony before the Subcommittees made it clear that one cannot now accurately predict the impact of the introduction of a particular organism on a new environment. It is possible to identify most of the parameters involved, study them, and, as Dr. Sharples stated, produce a rough "guesstimate" of the presence or absence of risk.[28] Estimates of the magnitude of any adverse reaction will be even less precise.

Testimony by both Dr. Sharples and Dr. Alexander bore directly on the problems associated with identification and estimation of potential impacts. Dr. Alexander pointed out that five components of risk associated with the release of organisms with new and novel genotypes could be identified. According to Dr. Alexander, the probability of each component occurring ranges from zero to one hundred percent. These risk components are: (1) the chance of release into a natural environment; (2) the possibility that an organism will survive there; (3) the likelihood that it will grow in that environment; (4) the possibility that it will make contact with species that it can injure; and (5) the chance that it will be harmful.[29] Dr. Alexander pointed out that "[i]f the new life form is deliberately released to perform some useful function, and it does what is expected of it, only the last two factors need be considered; that is, will it come in contact with a species it can harm and will it bring about deleterious change."[30] Such a change may also be an unanticipated result of the growth of the organism as it performs its designated function.

Both Dr. Sharples and Dr. Alexander rejected the argument that new genotypes released will not survive or grow because the engineered genetic changes in the organisms will place them at a disadvantage. Both witnesses pointed out that organisms have frequently insinuated themselves into new ecosystems, both with and without harmful effects.[31] Dr. Sharples specifically noted the example of the chestnut blight, which has virtually eliminated mature chestnut trees from their original niche, while young trees are not affected.[32]

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[28] Sharples testimony p.51.
[29] Alexander testimony p.13.
[30] Alexander testimony p.14.
[31] Alexander testimony p.15-16; Sharples testimony p. 22-23.
[32] Sharples testimony p.24. A semi-quantitative analysis of the introduction of foreign organisms was recently published by Daniel Simberloff. Simberloff noted that in 854 documented cases of exotic introduction, 71 of the cases resulted in the extinction of a natural population. It is important to note that the total number of cases (which would thus include those situations in

(Footnote continued)

Dr. Sharples also addressed the question of whether a genetically engineered organism released into a new ecosystem would produce significantly different results from those caused by translocations of natural organisms. In Dr. Sharples' opinion, the risks posed by genetically engineered organisms are similar to those presented by natural organisms. As she stated:

> I believe that the likelihood of an organism becoming established in a given environment is an ecological question regardless of the origin or nature of the differences that makes such an organism new or novel. The proportion of the genome which is "new" is not necessarily correlated with the degree of impact an organism can have on an ecological system. I believe that the analogy between recombinant organisms and introduced species is a valid one.[33]

Thus, although highly modified organisms can be produced, the interaction between the recombinant organism and an ecosystem may proceed in a manner similar to that which occurs for natural organisms.[34]

It was also noted that the impact of an organism on the environment could depend on the technique by which it was produced. Dr. Lindow contended that the organisms which he hopes to test in the field would be more stable than other engineered organisms. Dr. Lindow asserted that since the entire gene has been removed, his engineered organisms could not revert to their original type. In this experiment, he removed the ice nucleation gene specifically and did not cause any other change or addition

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[32] (continued)
which the novel organism did not survive) is not known; the number has been estimated to be many thousands, however. It is also important to note that extinction of a "natural population" is not the only possible negative outcome (e.g. Gypsy Moth). See Appendix A to this report.

[33] Sharples written testimony p.6.

[34] Mr. Clay, however, noted that new genetically engineered organisms may raise questions and present risks different from those posed by naturally occurring organisms. As he stated, "Genetic technologies for the first time allow the recombination of genes from organisms that could not or would not recombine in nature; this leads to the development of novel organisms. A number of theoretical risks associated with these organisms have been proposed by expert scientists. These concerns have been acknowledged and addressed not only by EPA, but also at the international level by committees of the World Health Organization and the Organization for Economic Cooperation and Development. Draft statements from both of these committees have recommended

(Footnote continued)

to the overall genome. In contrast, other techniques (such as protoplast fusion, ultra-violet or chemical treatment) involve the large scale alteration of existing DNA or the addition of large amounts of foreign DNA. Thus, in Dr. Lindow's opinion, no possibility exists that unexpressed genes carrying potentially adverse characteristics are present in his organisms.[35]

Despite Dr. Lindow's assurances, it is important to realize that the selection of organisms for further use is based on the presence of particular attributes sought by the researcher. No attempt is made to screen for other attributes of the organism unrelated to the particular manipulated gene that could have significant effects on the environment. As a result, the potential overall effect of the modified organism on its surroundings is not given sufficient consideration. The work of Giles and Whitehead, [1975] (cited earlier) provides an example of unanticipated characteristics that can be produced when two nonpathogenic microorganisms are combined. This work underscores the need to develop valid tests for the effects of novel organisms introduced into ecosystems.

Dr. Alexander testified that the probability of an organism's ability to grow and survive could easily be estimated, and possibly without great cost. He pointed out, however, that the identification and quantification of deleterious effects -- the key question -- would be much more difficult to address.[36]

In order to accumulate a data base on the behavior of genetically modified organisms in the environment, several witnesses suggested that limited scale field trials be permitted, especially with plants. Trials with plants were considered much easier to control than those for microbial experiments. The use of microcosm techniques was suggested for estimating microbial effects on ecosystems.[37]

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[34] (continued)
that careful assessment of potential risks be made." Response of Mr. Clay to supplemental written questions in Appendix B to the hearing transcript.

[35] Lindow testimony p.70-71.

[36] Alexander testimony p.55. Some witnesses, especially Drs. Kendrick and Sharples, suggested that the question is ecologically oriented and independent of the source of the organism. They contended that the impact of an organism is more a function of the relationship between its characteristics and its surroundings than of its origin. As noted, however, Dr. Lindow asserted that the probability that a given recombinant organism could affect the environment is at least in part related to the treatment of its DNA content.

[37] See, e.g., Hardy testimony p.82; response of Mr. Clay to

(Footnote continued)

As the testimony at the hearing demonstrated, a definite need exists to develop valid tests for assessing the effects of novel organisms on ecosystems in order to predict the relationship between the low probability and high consequence risk factors associated with each deliberate release. The tests should be capable of dealing with novel organisms from any source and must be based on sound ecological principles. The basic question is the relationship between newly introduced organisms and the environment that they will encounter. Thus, data gathered in an area with conditions highly dissimilar to the intended release area may not be suitable for decisionmaking. Genetically oriented scientists should be involved to determine if there are specific problems associated with organisms produced by any of the various techniques broadly covered by the heading "genetic engineering". Scientists with expertise in ecology and the environmental sciences must also be involved to ensure that all possible effects -- both positive and negative -- on the environment are adequately assessed.

C. Regulatory Framework

Issue:

Is the existing regulatory framework adequate to assess the risks and benefits associated with the deliberate release of a genetically engineered organism and thereby permit the benefits of the technology to be realized while minimizing the risk of an adverse environmental impact?

Findings:

Based on our investigation of the existing oversight mechanisms for biotechnology, it is clear that the current regulatory framework does not guarantee that adequate consideration will be given to the potential environmental effects of a deliberate release. No single agency or entity presently has both the expertise and authority to properly evaluate the environmental implications of releases from all sources. Although some federally funded researchers and commercial biotechnology firms presently request approval from the NIH RAC for deliberate releases, the RAC's ability to adequately evaluate the environmental hazards posed by deliberate releases is limited by both its expertise and its jurisdiction. The scope and content of EPA's regulatory effort is, at present, unknown, and that agency's

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[37] (continued)
supplemental written questions in Appendix B to the hearing transcript. Microcosm techniques involve establishing a small, closed system in an attempt to simulate field conditions.

ability is limited by its minimal experience in genetic engineering matters and unresolved questions concerning jurisdiction under TSCA. USDA may have jurisdiction to oversee deliberate releases under several statutes, but USDA's role is unclear because of its limited experience and disinclination toward oversight in this area.

Discussion:

The testimony presented to the Subcommittees made it clear that the responsibility for estimating risks and benefits from deliberate releases is divided between three agencies -- the NIH, the EPA, and the USDA -- with no agency having clear-cut authority to oversee this aspect of genetic engineering technology. Moreover, no agency currently has the expertise to consider properly the environmental issues raised by a deliberate release. Although the NIH RAC has considerable expertise in genetic engineering matters, it has only limited experience in addressing environmental considerations. Similarly, while EPA is experienced with environmental issues, it has never been involved with genetic engineering. The USDA, while it has examined some aspects of genetic engineering in plants, has had only very limited involvement with the technology. Consequently, no mechanism presently exists whereby the environmental implications of deliberate releases can be fully examined.

National Institutes of Health. Through its Recombinant DNA Advisory Committee (RAC), the NIH has been significantly involved in overseeing genetic engineering since the mid-1970s. The NIH RAC currently is the only federal entity with active responsibility for evaluating risks associated with research-scale field work. The RAC's limited jurisdiction, focus, and membership, however, make it ill-equipped to address the environmental risks posed by large-scale releases by commercial biotechnology firms. It is also not prepared to cope with the question of confidential business information or with the number of applications for releases anticipated.

The RAC is principally concerned with questions of biosafety under laboratory conditions for a specific type of recombinant DNA experiments[38] applicable to NIH funded situations only. In June 1976, the RAC released its first set of guidelines to govern recombinant DNA activities. These guidelines were, and still are, voluntary for non-NIH federal research and classify different types of experiments into a number of categories.[39] Since the time of their initial release, the guidelines have been relaxed until most -- over 90 percent -- of proposed experiments are exempt from them. Thus, most recombinant DNA experiments can be -- and are -- conducted without the necessity of prior RAC approval.[40]

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[38] Recombinant DNA experiments are defined by the RAC charter as involving removal, cutting, splicing, and reinsertion of RDNA using in-vitro techniques. See 48 Fed. Reg. 24557 (1983) (Part II).

Until recently the NIH guidelines prohibited releases of any type.[41] The RAC is now making an effort to become less involved with the deliberate release issue, however, at least with regard to genetically engineered plants. In April 1983, the NIH issued new relaxed guidelines addressing field tests of genetically modified plants.[42] Under the revised guidelines, researchers no longer have to apply for a formal exemption to conduct deliberate plant releases. Instead, they can proceed with field tests as long as they have the approval of their local Institutional Biosafety Committee (IBC) and a small "plant working group" set up within the RAC.[43] The NIH's general prohibition against deliberate releases for other types of organisms, except with the approval of the entire RAC, remains in effect. There appears to be strong momentum within the RAC to maintain its guidelines for deliberate releases generally and to be involved in overseeing biotechnology.

Despite the desire of the RAC membership to continue their involvement with deliberate releases, the RAC guidelines themselves are inadequate for dealing with the overall question of environmental impacts. The greatest problem is one of jurisdiction.[44]

By its charter, the RAC is specifically limited in its

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[39] Federal Register, July 1976, Part II, p.27902-27943. See written testimony of Mr. Karny.

[40] The Director of the NIH actually makes the final decision about any particular release. The RAC makes a recommendation to the Director, based on its review of the proposal, and the Director then accepts or rejects that recommendation.

[41] Talbot testimony p.115. The original NIH guidelines delineated several classes of experiments that were "not to be performed." Included in this list was the "deliberate release into the environment of any organism containing a recombinant DNA molecule." Gradually, this blanket prohibition was changed to permit certain releases with the approval of the RAC and the local Institutional Biosafety Committee (IBC). As discussed in the text, this provision has now been further relaxed, at least for plants. See Talbot testimony p.115-116.

[42] 48 Fed. Reg. 24548-49 (1983) (Part II).

[43] Id.; Talbot testimony p.116.

[44] Regarding the NIH guidelines, the OTA report stated: "In many respects, the Guidelines do not address the full scope of the

(Footnote continued)

authority to a narrow range of genetic exchanges using only techniques involving extraction and chemical treatment of DNA and to organisms altered using in vitro techniques.[45] As a result, many organisms and exchanges are by definition exempt from RAC review.

Moreover, the RAC's legal jurisdiction is limited to research activities funded by the NIH, and thus the RAC has no authority over endeavors by commercial biotechnology firms.[46] Private companies or groups that do not receive government research funds are not bound by the guidelines and have no legal obligation to seek RAC approval before conducting a deliberate release. This aspect of the RAC's limited jurisdiction makes it particularly incapable of guaranteeing that it can safeguard the environment from the adverse effects of deliberate releases. It is private industry, not research institutions, that has the greatest desire to move ahead with the release of new organisms on a large scale in order to achieve the broadest commercial applicability of their new genetically engineered products. Given the highly competitive nature of the biotechnology industry, it is not unlikely that some companies may decide not to request RAC approval out of a concern that the approval process could cause costly delays in testing and marketing their products. As Mr. Clay pointed out,

> In the long run ... the intensely competitive nature of the biotechnology industry will presumably create strong incentives for companies to reduce their costs, including those associated with developing strong safety programs. Many companies might establish outstanding safety programs even without federal oversight, but we cannot reasonably expect that economically vulnerable companies will voluntarily place safety as a high priority when their economic success may be at stake.[47]

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[44] (continued)
risks of genetic engineering. They cover one technique, albeit the most important; they do not address the admittedly uncertain long-term cultural risks; they are not legally binding on researchers receiving funds from agencies other than NIH; and they are not binding on industry." OTA Report, supra note 2 at 217.

[45] Dr. Chakrabarty pointed out that because his work involved "little recombinant DNA technology, and no cloning of eukaryotic genes" he would not be required to comply with the NIH guidelines, or any other guidelines, if he decided to test his microbes in the field. See response of Dr. Chakrabarty to supplemental written questions in Appendix B to the hearing transcript.

[46] Talbot testimony p.117-118.

[47] Response of Mr. Clay to supplemental written questions in
(Footnote continued)

In asserting the RAC's ability to oversee commercial activity, Dr. Talbot pointed out that while "there is no national law making the NIH guidelines mandatory for private industry," New York State and a number of other localities have passed local laws making the guidelines mandatory.[48] In addition, there is "a section of the guidelines labeled 'voluntary compliance' which encourages commercial organizations to comply voluntarily with the guidelines."[49] It must be noted, however, that only fifty-two[50] of the over 300 biotechnology firms now in existence have Institutional Biosafety Committees (IBC) that are registered with the RAC.

Moreover, even when private firms have come to the RAC for review of their proposals, confidentiality has been provided for those requesting such treatment in the past. Essentially all information connected with a proposal for a deliberate release has been considered confidential when such a request has been made.[51] Such a procedure makes it difficult to assure the public that adequate consideration is being given to the potential hazards posed by an experiment.

It is also important to note that the NIH guidelines themselves are not designed to facilitate consideration of the potential environmental effects of a genetically engineered organism. The guidelines are containment-oriented; that is, they are intended to prescribe conditions for laboratory experiments to prevent the accidental escape of an organism. As such, the guidelines do not ensure adequate protection of the environment from a deliberate release.

In addition to the problems presented by the scope of the NIH guidelines and the RAC's limited jurisdiction, the composition of the RAC prevents it from ensuring that environmental questions

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[47] (continued)
Appendix B to the hearing transcript. Dr. Sharples agreed with this assessment. She stated: "If there are no decisions and no structure is set up to accomodate [sic] or allow field testing, I think we will begin to see commercial firms eventually bypassing the RAC and pursuing product development without benefit of Federal oversight." Response of Dr. Sharples to supplemental written questions in Appendix B to the hearing transcript.

[48] Talbot testimony p.117.

[49] Talbot testimony p.118.

[50] Talbot testimony p.118.

[51] The RAC most recently afforded confidentiality to the proposals of Cetus Madison and Biotechnica at its September 19, 1983 meeting. Information pertaining to the proposals was generally kept secret and that portion of the RAC meeting during which the proposals were discussed was closed to the public.

receive adequate treatment. No ecologist currently serves on either the RAC or the RAC's plant working group.[52] Although some members of the RAC may have some sympathy for environmental considerations, none is specifically trained in the environmental or ecological sciences.[53] The RAC may well discuss the potential environmental implications of a deliberate release, but it does not posess the expertise to adequately evaluate those implications.[54] Thus, while a proposal for the deliberate release of microbes may have to be reviewed by the IBC, the RAC, and the NIH, there is no assurance that the proposal will be examined from an ecological perspective.

Further, the review procedure itself employed by the RAC to evaluate proposals for deliberate release has serious shortcomings. Addressing the "basic informality" of this procedure, Dr. Sharples stated that "[t]here is, as yet, no standardized method for evaluating requests for field testing and no established criteria for what information is needed to make a decision."[55] The procedure requires applicants to submit proposals at least 30 days before the meeting date. The RAC, however, does not specify the type or depth of material required.[56] As a result, applications are sometimes submitted with incomplete data, either because the data may not be available or because it is not considered important by the applicant. General requests in the past have ranged from one-page submissions to multi-page documents with numerous references.[57] There are no formal reports on the part of the RAC members to indicate the effort expended familiarizing themselves with the material prior to the meeting. Moreover, confidential information is not circulated prior to the meeting.[58]

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[52] Talbot testimony p. 137. The RAC membership list is included in Dr. Talbot's testimony in the hearing transcript.

[53] Talbot testimony p. 137. See response of Dr. Sharples to supplemental written questions in Appendix B to the hearing transcript.

[54] Dr. Talbot argued that environmental considerations are discussed by the RAC when it reviews a request for a deliberate release. Dr. Talbot related that in considering a release, RAC members "carefully consider... the possible hazards of the experiment." He pointed out that some members of the RAC propose "scenarios" of possible environmental damage and other members reply to them. Talbot testimony p.116. As noted in the text, however, the staff does not disagree with the statement that the RAC does make an effort to discuss the potential environmental risks of releases. The concern is that the RAC is not equipped to adequately evaluate those risks, even though it is recognized that the RAC does have some environmental expertise available to it. The RAC can seek advice from other agencies, as USDA and EPA, which have liaisons at the RAC meetings. These are non-voting

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The RAC does have three opportunities in the review process to request more information should the initial submission be inadequate: (1) the Executive Secretary of the RAC can request more information upon his review of the submission when it is received; (2) the RAC itself can return the proposal to the originator for additional information and request specific data; and (3) the Director of the NIH can return the proposal to the RAC requesting further information or clarification.[58] All of these procedures have been invoked in the past.[59] In fact, when two commercial applications for deliberate releases were submitted to the RAC recently, additional information was requested from both applicants.

Finally, because the RAC is in no way intended or designed to be a regulatory body, its procedures lack many of the safeguards built into other administrative decisionmaking processes. Judicial appeal and review of RAC decisions is not available -- at least not to commercial companies -- since the decisions are not binding on them. Despite being nonbinding, the RAC decisions do seem to have a certain precedential quality, especially if a request for a release is denied. The absence of outside review could pose problems for a company that is denied approval and thus suffers the "stigma" of that denial, despite its legal irrelevancy. Relatedly, there seemingly are no standards to guide RAC's decisionmaking process. As Dr. Sharples noted, there is no standardized method for evaluating a proposal for a deliberate release. Moreover, while other agencies are bound by the requirement that their decisions not be "arbitrary or capricious" and that they have some basis in a factual record, the RAC is subject to no such requirement -- again, because its decisions are

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[54] (continued)
representatives, however, and the concern is that the voting membership of the RAC include experts in the environmental sciences. The RAC also has the authority to avail itself of additional expertise in the form of consultants as required. In this manner it could bring specific ecological expertise to bear on these problems, although it has not done so to date.

[55] Response of Dr. Sharples to supplemental written questions in Appendix B to the hearing transcript. See also Karny testimony p.38.

[56] Response of Dr. Sharples to supplemental written questions in Appendix B to the hearing transcript. See also Karny testimony p.37.

[57] Karny testimony p.37.

[58] Staff conversation with Dr. William Gartland, NIH Office of Recombinant DNA Activities. Material is only circulated to RAC members who certify that confidentiality will be maintained.

[59] Staff conversation with Dr. Gartland; written testimony of Mr. Karny.

not binding on a commercial firm.[60]

Environmental Protection Agency. Although it has general responsibility within the Federal Government for protecting the environment, EPA has not historically been involved in the regulation or oversight of genetically engineered substances or organisms. Recently, however, EPA decided to become active in seeking ways to regulate deliberate releases of genetically engineered organisms into the environment. Here, too, however, the question of jurisdiction presents problems for oversight.

Two major statutes affect EPA's jurisdiction and regulatory authority in this area. One is the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)[61]; the other is the Toxic Substances Control Act (TSCA).[62]

Under FIFRA, the EPA Office of Pesticides Programs (OPP) appears to have clear jurisdiction to regulate the use of pesticides consisting of genetically engineered organisms. In fact, the OPP is currently developing regulations for such pesticides."[63] Under FIFRA, EPA will require companies to provide extensive data on the biological fate of their commercial organisms; this will enable the agency to develop a sufficient basis for accurately forecasting potential environmental risks.[64]

EPA's jurisdiction to regulate other aspects of biotechnology that do not involve pesticides is less clear. At the Subcommittees' hearing, Mr. Don Clay stated that EPA intended to exercise authority under TSCA to regulate biotechnology and would subsequently proceed to develop regulations governing deliberate

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[60] Even if the RAC's decisions were binding on commercial firms, the RAC still would not be subject to such requirements. The RAC is not subject to the Administrative Procedures Act. 5 U.S.C. S 5 U.S.C. 1, S 3(3): Lombardo v. Handler: 397 F supp., 792 (DUC 1975), aff'd 546 F 2nd 1043, (D.C. Cir.) cert. denied, 431 US 932.

[61] 7 U.S.C. S 136-136y; Clay testimony p.121; Karny testimony p.37.

[62] 15 U.S.C. S 2601-2629; Clay testimony p.121; Karny testimony p.37. Other statutes, such as the Clean Water Act, the Resource Conservation and Recovery Act, and the Clean Air Act, may provide EPA with authority to regulate various aspects of deliberate releases, depending on their purpose and possible effect. FIFRA and TSCA are the two principal statutes that define EPA jurisdiction here, however, and the discussion is limited to them.

[63] Clay testimony p.122. At the hearing, Mr. Clay stated that EPA anticipates submission of the first applications for registration or for an experimental use permit for a genetically engineered pesticide within 18 to 24 months. Mr. Clay contended that EPA has significant experience under FIFRA in dealing with naturally occurring microbial entities that act as pesticides. In his

(Footnote continued)

releases.[65] Whether the EPA can in fact regulate biotechnology under TSCA remains to be seen, however, for while TSCA provides EPA the authority to require notification of and then to regulate "chemical substances" that pose potential risks to health or the environment,[66] the act does not specifically address the question of new genetically engineered organisms.[67]

Despite the uncertainties concerning its authority under TSCA, the agency is preparing to assume a regulatory role. The agency's Office of Research and Development has conducted seminars over the past year to educate agency staff about widely used industrial microorganisms and their potential hazards, and the Administrator's Toxic Substances Advisory Committee (ATSAC) has been studying issues related to this area since last September.[68] Several research projects designed to enhance EPA's understanding of the potential environmental impacts of releases are also underway.[69] Moreover, in March of this year, a draft report entitled "Regulation of Genetically Engineered Substances Under TSCA" was prepared by the Office of Toxic Substances that concluded that a strong argument could be made for EPA jurisdiction under TSCA.[70] The same conclusion was put forward in a March 14, 1983, memorandum from the Office of the General Counsel to Dr. John Todhunter, who was then the Assistant Administrator for Pesticides and Toxic Substances.[71]

The arguments in favor of TSCA coverage of genetically engineered organisms are relatively straightforward. The statutory argument is that any life form is composed of "chemical substances" as defined in TSCA and that life forms themselves can be considered "chemical substances" under TSCA because they are

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[63] (continued)
opinion, this experience will greatly facilitate EPA's evaluation of proposals for deliberate releases under FIFRA. Mr. Clay noted, however, that EPA has no experience yet with genetically engineered products. *Id.*

[64] Clay testimony p.122-123. Mr. Clay pointed out that EPA's Office of Pesticides Programs (OPP) has extensive regulations addressing non-genetically engineered microbial pesticides. In Mr. Clay's view, "These standards, which require product analysis, human safety, and ecological effects data, are adequate for the assessment of non-engineered pesticides. *Id.* at 123. Mr. Clay conceded, however, that "more extensive data or information may be necessary to evaluate genetically engineered pesticides." Nevertheless, Mr. Clay believed that "the regulation establishing these data requirements explicitly provides the necessary flexibility to require additional data if OPP determines a need for it." *Id.*

[65] Clay testimony p.123-125, 128.

[66] 15 U.S.C. S 2601(b)(1)-(3).

"combinations of organic substances that occur in nature or that occur in whole or in part as a result of a chemical reaction", as stated under the statute.[72] A policy argument is also made that because TSCA was intended to be a "gap filler" when enacted by Congress, it is appropriately invoked here to cover areas untouched by other statutes.[73]

On the other hand, an argument exists against TSCA jurisdiction, based principally on a stricter construction of the language and statutory history of TSCA, and the strength of this argument is acknowledged by EPA.[74] The argument is made quite simply that no indication whatsoever exists that Congress intended TSCA to cover genetically engineered life forms. It is argued that Congress never even considered this possibility when enacting TSCA and that to apply TSCA to biotechnology would distort its purpose. An argument is also made that genetically engineered organisms are not "manufactured" in the same sense as inanimate substances and thus the provisions of TSCA are not easily applicable to these organisms.[75] In addition, TSCA specifically states that its application is not to hinder innovation.[76]

If TSCA is applicable to genetically engineered organisms, EPA would utilize section 5 of the Act to require biotechnology companies to submit Premanufacturing Notices for EPA review before a genetically engineered organism could be produced and then released into the environment.[77] Under this procedure, a company must notify EPA of its intent to put a new "chemical substance" into production. If EPA determines that the product may pose a significant risk to the public health or the environment, the agency can prevent its production.[78] TSCA places a greater burden on EPA than does FIFRA to prove the existence of such a risk before it can block production of a substance, but Mr. Clay asserted that EPA is confident of its ability to assess any potential environmental difficulties. As Mr. Clay explained:

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[67] Clay testimony p.124; Karny testimony p.38. See generally the EPA internal report, entitled "Regulation of Genetically Engineered Substances Under TSCA," in Appendix (B) to this report.

[68] Clay testimony p.138.

[69] Clay testimony, p.126-127.

[70] See Appendix B to this report.

[71] See EPA memo in Appendix C to this report.

[72] 15 U.S.C. S 2602(8).

[73] See EPA report in Appendix B; EPA memo in Appendix C; Karny testimony p.38.

[74] See EPA report in Appendix B; EPA memo in Appendix C.

[75] See EPA report in Appendix B; EPA memo in Appendix C; Karny testimony p.38.

[76] TSCA section 2. 15 U.S.C. S 2601(b)(3).

[77] Clay testimony p.124. 15 U.S.C. S 2604.

[78] 15 U.S.C. S2604. Karny testimony p.40-41.

> Under FIFRA, we have to have the data that we have before we will register it, and you have to have it registered before you can use it as a commercial application or receive an experimental use permit. Under TSCA, the burden is more on the Government to prove there is a problem. TSCA is a notification scheme where you must notify the agency 90 days in advance to manufacture a new chemical substance, which is defined as something not already in existence or on the inventory. In that case what we have to do, we would have to make a finding that there would be a possibility of significant risk in order to do it, but we think we can do so. We either have significant risk or we have exposure to do that, and then require the testing that way. There is more burden on the agency under TSCA than there is FIFRA. No question about that. However, we think that the TSCA is designed as a very flexible statute and that we have flexibility to insure the testing is done.[79]

Because EPA only recently made the final decision to proceed under TSCA, its risk/benefit activities are at a very preliminary stage. The Agency has clarified its authority, internally, under the TSCA and will regulate DNA-containing commercial products in the Office of Toxic Substances (OTS). At the time of this writing, however, the agency has not clearly defined the specifics of its policy. Nor has EPA defined the scope of its regulatory activity toward biotechnology. As Mr. Clay stated at the hearing, "[T]here is considerable uncertainty concerning which, if any, areas of recombinant DNA technology will require regulatory controls and what these controls should be."[80]

Products identified as relating to agriculture in the form of insecticides, fungicides, or rodenticides fall under the Federal Insecticide, Fungicide and Rodenticide Act which is administered by the EPA Office of Pesticide Programs,[81] and the OPP has published guidelines which can be applied to registration of pesticides involving novel organisms intended for deliberate release.[82] The OTS, however, is only beginning to formulate its approach under TSCA to deliberate releases.

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[79] Clay testimony p.140.

[80] Clay testimony p.125-126.

[81] Because of their unique nature, however, new genetically engineered organisms may potentially be covered by several statutes. Which statute best applies may well depend upon the use to which the particular organism is to be put, rather than its actual characteristics. For example, EPA was initially uncertain whether the ice nucleation bacteria developed by Dr. Lindow would be covered by FIFRA or by TSCA. EPA recently concluded that FIFRA is the more appropriate statute, however, because the bacteria "exert ... a pesticide action" and their intended use is for their "pesticidal effect." See Memorandum from Mr. Stanley H. Abramson,

(Footnote continued)

Moreover, because EPA has never been involved in overseeing genetic engineering activities, significant efforts will need to be made to familiarize EPA personnel with the various aspects of the technology. Mr. Clay related EPA's need to "educate existing staff [and] recruit new expertise as is needed."[83] He also stressed the need to "develop methodologies to evaluate environmental fate, human and environmental exposure, and potential environmental and health hazards of genetically engineered organisms," and called for coordination with other agencies and groups.[84]

Department of Agriculture. The hearing record upon which this report is based does not provide much insight into USDA's jurisdiction over biotechnology or the agency's intentions toward deliberate releases in the future. The role of USDA in overseeing biotechnology can best be described as unclear. USDA has been involved in recombinant DNA activities to some extent, but unlike the NIH and EPA, the agency has not made an effort to regulate in this area. USDA potentially has authority to regulate the deliberate release of genetically engineered organisms. That authority may be limited, however, by USDA's focus on matters affecting agriculture and by the requirement in most pertinent legislation that an activity be involved in interstate commerce before it comes within the agency's jurisdiction.

Several statutes arguably provide USDA with the ability to control deliberate releases. USDA's most significant potential jurisdiction appears to be with regard to genetically modified plants. Three laws may apply here: (1)the Plant Quarantine Act (PQA); (2)the Plant Variety Protection Act (PVPA); and (3)the Federal Noxious Weed Act (FNWA).

(1) Plant Quarantine Act -- The PQA[85] empowers the Secretary of Agriculture to control disease-carrying plants in three ways. First, the Act gives the Secretary authority to regulate the importation into the United States of any "nursery stock"[86] and to

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[81] (continued)
EPA Associate General Counsel, to Mr. Don Clay, in Appendix C to this report.
[82] 47 Fed. Reg. 53192 (1982).
[83] Clay testimony p.128.
[84] Clay testimony p.126-127.
[85] 7 U.S.C. S 151-167.
[86] The Act defines "nursery stock" as "all field-grown florists'
(Footnote continued)

prohibit its entry unless it is "free from injurious plant diseases and insect pests."[87] Second, the Act authorizes the Secretary to restrict the importation of plants other than those defined as nursery stock if their introduction "may result in the entry ... of injurious plant diseases or insect pests."[88] Third, the Act enables the Secretary to prevent the interstate movement of any nursery stock or other plants "of any character whatsoever" that are "capable of carrying any dangerous plant disease or insect infestation" that is "new to or not theretofore widely prevalent or distributed within and throughout the United States."[89]

As is apparent, the PQA grants USDA broad powers to restrict the introduction of dangerous plants into the country and to limit their movement between States. The terms of the Act apply to virtually every type of plant and as such, may be broad enough to include any new plant created through genetic engineering. If so, the Secretary would have the ability to control the movement of genetically engineered plants into or within this country.

While the Act gives USDA broad general jurisdiction, the agency's specific power is not without limitations. First, to be covered by the Act, a plant must be one that is capable of carrying a plant disease. It is entirely possible that a new genetically engineered plant may be capable of causing a good deal of environmental damage but not be considered a carrier of "disease." Second, the Act empowers the Secretary to control only the importation or interstate movement of disease-carrying plants. This could limit USDA's ability to regulate deliberate releases.

(2) Plant Variety Protection Act -- The PVPA[90] establishes protection for "novel varieties" of sexually reproducing plants if they are sufficiently unique in the determination of the Secretary of Agriculture.[91] This protection essentially amounts to an agricultural patent and prevents other "breeders" of plants from replicating a plant,[92] unless the widespread usage of the plant is deemed to be in the national interest.[93] In order to apply for

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[86] (continued)
stock, trees, shrubs, vines, cuttings, grafts, scions, buds, fruit pits, and other seeds of fruit and ornamental trees or shrubs, and other plants and plant products for propagation, except field, vegetable, and flower seeds, bedding plants, and other herbaceous plants, bulbs, and roots." Id. S 152.

[87] Id. S 154.

[88] Id. S 159.

[89] Id. S 161.

[90] 7 U.S.C. S 2401 et seq.

[91] Id. S 2401.

[92] Id. S 2402.

[93] Id. S 2404.

such protection, the developer of a new plant must provide USDA with detailed information on the plant. If recombinant DNA techniques are utilized in the creation of the plant, that information would be included on the application for protection and would alert USDA that a deliberate release might be contemplated by the breeder.[94]

Whether this knowledge would do any real good is unclear, however. The PVPA does not grant USDA any power to prevent a release -- or to prevent anything for that matter. The Act seems simply to provide a means for the granting of protection to new plants. The only real power on the part of USDA under the Act is to grant or deny that protection.

(3) Federal Noxious Weed Act -- The FNWA[95] may provide USDA with its broadest authority to regulate deliberate releases. The purpose of FNWA is to control "noxious weeds" that could have "adverse effects upon man or his environment" in order to protect agriculture and commerce in this country.[96] "Noxious weed" is defined very broadly in the statute as

> any living stage (including but not limited to, seeds and reproductive parts) of any parasitic or other plant of a kind, or subdivision of a kind, which is of foreign origin, is new to or not widely prevalent in the United States, and can directly or indirectly injure crops, other useful plants, livestock, or poultry or other interests of agriculture, including irrigation, or navigation or fish or wildlife resources of the United States or the public health.[97]

Under FNWA, anyone wishing to "distribute" any noxious weed in interstate commerce must request the approval of the Secretary of Agriculture. The Secretary can deny approval for distribution if dissemination of the weed "may reasonably be expected to have to a serious degree any effect specified."[98]

FNWA's definition is seemingly broad enough to encompass genetically engineered plants, and the regulatory structure instituted by it would enable USDA to oversee requests for deliberate releases of these plants. Like the other statutes, however, FNWA provides USDA with jurisdiction only over plants distributed in interstate commerce. If the genetically modified plants did not move in interstate commerce, USDA could not regulate them under FNWA.

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[94] Kendrick testimony p.135.
[95] 7 U.S.C. S 2801-2813.
[96] _Id._ S 2801.
[97] _Id._ S 2802(c).
[98] _Id._ S 2809.

In addition to the three statutes governing plants, there are laws that may provide USDA with the authority to regulate genetically engineered microorganisms and animals as well. One significant act is the Federal Plant Pest Act (FPPA).[99] The FPPA forbids the importation or interstate movement of any "plant pest" without the approval of the Secretary of Agriculture. FPPA defines a "plant pest" as any stage of any

> insects, mites, nematodes, slugs, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof, viruses, or any organisms similar to or allied with any of the foregoing, or any infectious substances, which can directly or indirectly injure or cause disease or damage in any plants or parts thereof, or any processed, manufactured, or other products of plants.[100]
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> This broad definition could be construed to include genetically engineered organisms of all sorts, excluding vertebrate animals and nonparisitic plants. In order to come within the purview of FPPA, however, an organism must be one that "can injure" plants. While this requirement may be theoretically applicable to most genetically engineered organisms, it could limit USDA's jurisdiction somewhat. For example, if a genetically engineered organism somehow did not pose a threat to plants, it would not fall within the jurisdiction of FPPA, even if it presented significant risks to other aspects of the environment.

Like the Federal Noxious Weed Act, FPPA addresses the sale, transportation, and release of new organisms, but it does not apply to their production. Under FPPA, the Secretary of Agriculture is to weigh the relative risks and benefits presented by the dissemination of an organism. If the Secretary determines that the risks outweigh the benefits, he can prevent the distribution of the organism. Because the risks addressed by FPPA pertain to the injury of plants, environmental questions necessarily must play some role in USDA's deliberations under the Act.

According to Dr. Kendrick, several other statutes may also provide some basis for USDA jurisdiction over deliberate releases. Under the Virus, Serum, and Toxin Act,[101] USDA oversees animal biological products that move in interstate commerce. Under this Act, USDA could control releases to the extent that these products are produced through recombinant

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[99] 7 U.S.C. S 150aa-150jj.
[100] *Id.* S 150aa(c).
[101] 21 U.S.C. S 151-158.

DNA techniques or other methods of genetic modification.[102] Additionally, under a variety of animal quarantine and related laws, USDA could conceivably regulate deliberate releases if the genetically engineered organisms could be classified as disease agents. These statutes empower USDA to prevent the introduction or interstate dissemination of contagions or diseases communicable to animals.[103]

In addition to whatever jurisdiction it has under the statutes just discussed, USDA may have authority to regulate deliberate releases under a little-known, and even less used, Executive Order. If applicable, this order could potentially give USDA broad power to regulate in this area.

Issued in May 1977, Executive Order 11987 generally prohibits the introduction of exotic organisms into the environment. Specifically, the Order directs executive agencies to restrict the introduction of exotic species into the environment in two respects.

First, the Order declares that "[e]xecutive agencies shall, to the extent permitted by law restrict the introduction of exotic species into the natural ecosystems on lands and waters which they own, lease, or hold for purposes of administration; and shall encourage the States, local governments, and private citizens to prevent the introduction of exotic species into natural ecosystems of the United States." Second, the Order states: "Executive agencies, to the extent they have been authorized by statute to restrict the importation of exotic species, shall restrict the introduction of exotic species into any natural ecosystem of the United States."[104]

Although the Exective Order thus sets forth a broad ban on the introduction of exotic organisms, it goes on to permit their introduction in certain cases. Specifically, the Order declares: "This Order does not apply to the introduction of any exotic species ... if the Secretary of Agriculture or the Secretary of the Interior finds that such introduction or exportation will not have an adverse effect on natural ecosystems."[105]

On its face, the Order seems to place great authority in the hands of the Secretary of Agriculture -- along with the Secretary of the Interior -- to regulate introductions of the type covered by the Order. It is unclear, however, whether this Order can be construed to apply to the deliberate release of genetically engineered organisms. If it does, the Order would provide USDA with significant authority to regulate deliberate releases.

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[102] Kendrick testimony p.134.

[103] Kendrick testimony p.133-134.

[104] See Executive Order 11987 in Appendix C to this report.

[105] See Appendix C to this report.

As the preceding discussion reveals, the jurisdiction of USDA over deliberate releases of genetically modified organisms is ambiguous and subject to interpretation. A number of statutes and even an Executive Order exist that arguably give USDA a basis for action in this area. These laws were not enacted with genetically engineered organisms in mind, however, and their applicability here is thus uncertain. Dr. Kendrick stated that "[t]he perception that the USDA has very limited authority to regulate new or modified organisms resulting from genetic engineering is correct."[106]

Even if the cited statutes and Order were relevant, their application may depend as much upon USDA's inclinations as upon their wording and intent. As mentioned earlier, USDA has not taken -- and does not seem predisposed to take -- an active role in oversight of biotechnology. USDA's involvement and expertise in genetic engineering is limited at best.

USDA, like the NIH, does have a Recombinant DNA Advisory Committee to oversee biotechnology research. Also, like the NIH, the USDA RAC has reviewed proposals for deliberate releases. Unlike the NIH RAC, however, the USDA RAC is not aggressive in its assertion of expertise in this area. The USDA RAC reviews requests for field tests of plants jointly with the NIH RAC and defers heavily to the judgment of the NIH RAC in its decisions. As Dr. Kendrick testified, the USDA RAC views the NIH RAC as "the lead agency for developing guidelines for conducting recombinant DNA research."[107]

If USDA maintains its present approach, it is unlikely that the agency will be able to provide the needed oversight of deliberate releases. Although it may have jurisdiction over a variety of genetically engineered organisms, USDA is not actively seeking to assert its potential authority. Even if USDA were to claim jurisdiction over deliberate releases, there would be some shortcomings in its ability to adequately protect the environment. As noted, each of the statutes that may provide USDA a basis of authority is limited in its applicability. Moreover, the USDA RAC suffers from a handicap similar to that of the NIH RAC: it has no specific ecologist or individual specifically trained in the environmental sciences among its membership. While the USDA RAC members may be sympathetic to environmental concerns, they are not specifically trained to assess them. In any event, the USDA RAC typically has deferred to the NIH RAC in reviewing proposals for deliberate releases. The shortcomings of the NIH RAC in overseeing releases have been previously discussed, and the USDA RAC's deference further compounds this problem.

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[106] Response of Dr. Kendrick to supplemental written questions in Appendix B to the hearing transcript. It must be noted, however, that USDA does not believe that the problems associated with genetically engineered organisms are markedly different from those posed by natural organisms. Consequently, USDA believes that any potential risks presented by these new organisms can be adequately handled under existing statutes and regulations, however limited they may be. Id.

[107] Kendrick testimony p.132.

Oversight Generally. As the above discussion indicates, the status of federal oversight over the deliberate release of genetically engineered organisms and the environmental questions relating thereto is uncertain and subject to dispute. No statute gives any agency express jurisdiction over commercial biotechnology.

The significant differences between the perspectives of the three agencies involved in overseeing this problem have resulted in a situation that does not provide assurance that the environment will be adequately protected. While the NIH, EPA, and USDA all believe that oversight over deliberate releases is important, each agency has limited the scope of its activities to coincide with its agency mission. Thus, the RAC has specialized on containment procedures and laboratory oriented safety; USDA has focused on plant pests and pathogens relative to food supplies; and until recently, EPA concentrated on agricultural pesticide products under FIFRA.

Additionally, the historical involvement of the NIH and USDA in genetic engineering matters has led those agencies to conclude that the current regulatory scheme is sufficient to protect against environmental harm, despite its significant shortcomings. Both Dr. Talbot and Dr. Kendrick testified that no new regulation was needed.[108] Clearly, however, some additional regulatory effort must be made to close the gaps that currently exist in oversight of the technology.

Further, no agreement exists that a group with sufficient experience in both the regulatory and scientific aspects of the problem is prepared to handle the anticipated flow of applications involving deliberate releases. As Mr. Karny asserted, the current "balkanized" regulatory structure may mean that proposals for releases will receive inadequate attention.[109] With both authority and expertise scattered among several agencies, it is doubtful that adequate consideration will be given to all requests for deliberate releases.

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[108] Talbot testimony p.141; Kendrick testimony p.136.
[109] Response of Mr. Karny to supplemental written questions in Appendix B to the hearing transcript.

Society has much to gain from the commercial applications of genetic engineering through deliberate releases, and the Subcommittees conclude that there is ample legislative basis for instituting a process to estimate the risk factors associated with such releases. The manner in which the respective agencies are limiting the scope of their activities, however, while understandable in terms of the specific mission of each agency, indicates that there may be areas that are not adequately examined for evidence of environmental risks.

Moreover, the ambiguity that currently surrounds the jurisdiction of the agencies -- especially USDA -- to oversee deliberate releases must be resolved and the relative authority of each agency must be clarified. This jurisdictional clarification is particularly important for USDA, given the vast potential authority of the agency. USDA could be a "sleeping giant" in the regulation of biotechnology, and if its role is not clarified, needless duplication of effort, if not outright conflict, could occur in the regulation of the technology.

It must be understood that the absence of good regulations addressing the matter of deliberate releases could actually hurt the biotechnology industry and deprive society of the benefits of deliberate releases. This is especially of concern in the immediate future. As Dr. Chakrabarty asserted, strong guidelines are needed to assure the public that deliberate releases approved under them will not present an unreasonable risk. If applications are received and approved without an in-depth examination by qualified experts, public concern over the possible dangers of deliberate releases could reach such a level that calls for a total ban on releases would be heard.[110] Moreover, as Dr. Sharples noted, a clearly defined, "smoothly operating" system of federal oversight could facilitate both development and marketing of genetically engineered products.[111]

The need for a procedure which will provide public assurance that the risk of adverse effects associated with these activities has been minimized is thus clear. Because the biotechnology industry is in its infancy, it is important that any oversight must be accomplished with sufficient built-in flexibility to assure that it will not hamper research and development in this area. It is equally important, however, that adequate oversight be established.

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[110] Already, a lawsuit has been filed that challenges the activity of the NIH RAC in approving proposals for deliberate releases. The suit was filed on September 14, 1983, by three organizations -- the Foundation on Economic Trends, Environmental Action, Inc., and the Humane Society of the United States -- and alleges that the NIH has failed to comply with the requirements of the National Environmental Policy Act in reviewing and approving requests for deliberate releases. Particularly, the suit points to the absence of any established methodology or protocols for adequately evaluating the risks posed by deliberate releases. See Foundation on Economic Trends v. Heckler, No. 83-(2714) (D.D.C. Sept. 14, 1983).

[111] Response of Dr. Sharples to supplemental written questions in Appendix C to the hearing transcript.

D. Recommendations

The following recommendations are based on the findings and discussion above:

(1) The EPA should proceed with its stated intention to extend its authority to include all deliberately released organisms not specifically identified as part of the legal obligation of another agency. In view of EPA's stated conclusion that the Toxic Substances Control Act (TSCA) does provide it with authority to oversee deliberate releases and the fact that Congress intended TSCA to be "gap filling" legislation, no additional legislation or clarifying amendments are needed at this time. EPA should, however, establish formal communications and agreements with other agencies to ensure that gaps and redundancies in the regulatory structure do not occur. A major goal should be to permit research and commercialization to proceed with minimum interference while adequately addressing environmental and public health concerns.

(2) Until such time as EPA's regulations are promulgated, an interagency task force should be established to review all proposals for deliberate releases. EPA should take the initiative in organizing this panel. The panel should be comprised of representatives from EPA, USDA, NIH, and any other appropriate federal agency or entity directly involved from either the scientific or regulatory perspective. The panel should establish an environmentally oriented risk/benefit assessment program to evaluate current proposals for deliberate releases and to provide a data base for decisions on future releases. The panel should also develop a uniform set of guidelines to govern deliberate releases. The panel should, moreover, serve the function of educating the public about the potential risks and benefits associated with this aspect of biotechnology. Consideration should be given to making this panel a permanent oversight body even after EPA has promulgated regulations to ensure that the broadest possible expertise is brought to bear in overseeing the technology.

(3) No deliberate release should be permitted by EPA, NIH, USDA, or any other federal agency until the potential environmental effects of the particular release have been considered by the interagency review panel. The panel shall consider the effects of any environmental release, regardless of size or intent. Each

agency should evaluate proposals for deliberate releases according to a uniform set of guidelines to be developed by the interagency task force. It is recognized that initially decisions may be made on the basis of incomplete data.

(4) The task force should consider the need for oversight of research scale releases and, if appropriate, develop guidelines for reviewing proposals for such releases. The task force should prepare a report containing its conclusions on this matter within 90 days of its establishment. The report should be made available to the Subcommittee.

(5) The NIH should cease its practice of evaluating and approving proposals for deliberate releases from commercial biotechnology companies. The NIH should review proposals only from parties engaged in NIH-sponsored research, and refer requests from industry to the appropriate agency.

(6) The NIH and USDA should revise the membership of their respective Recombinant DNA Advisory Committees (RAC) to include individuals specifically trained in ecology and the environmental sciences.

(7) The General Accounting Office should review the activities of USDA in overseeing biotechnology and evaluate the agency's authority to regulate deliberate releases under all relevant statutes, regulations, and executive orders.

Discussion:

As the preceding section related, the staff has determined that no mechanism presently exists to adequately review proposals for deliberate releases. Authority and expertise is scattered among at least three executive agencies, and as a result, no single body is capable at this time of evaluating genetically engineered organisms for their potential environmental impact.

As noted, the NIH RAC has considerable expertise in matters involving recombinant DNA, but no expertise in environmental concerns. Indeed, the RAC contains no one specifically trained in ecology or the environmental sciences among its membership. Moreover, the RAC's jurisdiction is limited only to activities funded by the NIH. Similarly, EPA has experience in assessing environmental risks, but has never been involved in oversight of recombinant DNA technology. The USDA, while it has a RAC, has not been especially active in evaluating organisms created through genetic engineering. Moreover, the parameters of USDA's jurisdiction over biotechnology are uncertain.

In light of the deficiencies in the expertise and jurisdiction of each agency, the staff has concluded that the best way to ensure that the environmental implications of deliberate releases from all sources are properly evaluated is to establish an interagency task force to review proposals for such releases. Such a task force should be comprised of representatives from EPA, NIH, and USDA, as well as any other appropriate federal agency or entity.

An interagency task force would bring the experience and expertise of the various executive agencies together in a single entity to evaluate deliberate releases and fill the gaps that currently exist in the review process. Not only would an interagency group combine EPA's environmental expertise with NIH's familiarity with molecular biology, it would also encompass the jurisdiction of EPA, NIH, and USDA, as well as any other agency involved in the task force. In this way, the broadest oversight possible over deliberate releases would be ensured.

The interagency task force would perform a number of essential functions. First, and foremost, the group would review requests for deliberate releases. Although each individual agency would collect the data necessary for evaluating the potential environmental effects of a proposed release, the interagency group would review the request and make a recommendation about the release. Second, the interagency task force would develop a uniform set of guidelines by which to evaluate requests for releases and develop an appropriate environmental risk assessment program. Because the biotechnology industry is evolving at a fairly rapid rate and new risk assessment data are constantly being developed, such guidelines must be flexible. The task force will ensure that the guidelines will be sufficiently flexible to accommodate new information. Moreover, the development of a risk assessment program would facilitate the review process and ensure that the environmental risks of a release are minimized. Third, the interagency group would develop a much-needed data base on the environmental implications of releases of novel organisms. Fourth, the group would perform a valuable role in educating the public about the benefits and risks of deliberate releases. The group would publicize both the facts of and basis for its decisions, and its meetings would be open to the public. The group's public-awareness role could include ensuring that all concerned researchers are aware of the review procedures. Fifth, and finally, the group would coordinate efforts and define the scope of each agency's activities to ensure that all potential environmental and public health impacts are considered before approving release into the environment.

In addition to these broader functions, it is recommended that, as an initial function, the task force assess the need for oversight of small scale releases. This report focuses primarily on large scale, commercially oriented releases. However, since organisms are known to inexorably expand if they encounter a favorable environment, the size of the release may not be the critical factor. It is possible that a research scale release could result in major environmental damage or adverse public health effects. The staff is concerned that such releases conducted as a result of non-NIH funded research would not be included within the scope of EPA's regulatory effort. The task force should consider the extent to which such releases would be subject to existing regulations and determine whether additional guidelines are needed. It is further recommended that the task force prepare a report, which should be made available to the Subcommittee, within 90 days, to ensure that this matter receives appropriate attention.

The task force would be particularly important in the immediate future, as EPA proceeds with its development of regulations for deliberate releases under TSCA. EPA estimates that its regulations will be developed "over the next few years"[112] and that at least a year and a half will be required for the necessary information-gathering and evaluative process to occur.[113] Until EPA completes this process and issues regulations, the current vacuum in oversight will persist. An interagency task force is needed to provide the necessary oversight during this interim period and to facilitate the transition to regulation by EPA.

The above comments should not be taken to imply that the task force is needed only as a temporary measure until EPA issues its regulations. To the contrary, consideration should be given to instituting the task force or a similar interagency group as a permanent review mechanism within the Executive Branch even after EPA has developed its regulations. It is unlikely that EPA will totally acquire the necessary expertise in matters involving recombinant DNA in the next few years, although it is anticipated that participation in the interagency task force would enhance EPA's understanding of the complexities of genetically modified organisms. A permanent interagency group to review proposals for deliberate releases would guarantee that appropriate attention is given to the environmental issues posed by a particular release. However, a decision about the permanency of the interagency group would best be made after the specifics of EPA's regulatory effort are known.

Ample precedent exists for the establishment of such an interagency group. An Interagency Advisory Committee was established in 1976 to coordinate activities among the various federal agencies that pertained to recombinant DNA technology.[114]

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[112] Response of Mr. Clay to supplemental written questions in Appendix C to the hearing transcript.

[113] Staff conversation with EPA.

[114] Response of Dr. Talbot to supplemental written questions in Appendix C to the hearing transcript. The purpose of the

(Footnote continued)

Although that committee has been dormant for the past few years,[115] its creation reveals that the agencies do recognize that the questions presented by genetic engineering transcend agency boundaries. The task force is to a great extent simply a modification of the concept embodied in the previous Interagency Advisory Committee.

Moreover, the need for better coordination between the exective agencies regarding recombinant DNA technology has been repeatedly emphasized over the years. In an exchange of correspondence in late 1978, then Health, Education and Welfare Secretary Joseph Califano, then Agriculture Secretary Bob Bergland, and then EPA Administrator Douglas Costle discussed their agencies' respective jurisdictions over the technology.[116] Mr. Califano requested that Mr. Bergland and Mr. Costle direct their agencies to review their regulatory authority in this area because "there may be significant recombinant DNA research conducted in the private sector" over which HEW itself had no jurisdiction.[117]

More recently, in February 1983, Dr. John Todhunter, formerly EPA's Assistant Administrator for Pesticides and Toxic Substances, and Dr. Courtney Riordan, then EPA's Acting Administrator for Research and Development, jointly wrote Dr. William Gartland, NIH's Director of Recombinant DNA Activities, and called for both greater attention to environmental issues and increased cooperation between EPA and NIH in this area.[118] Drs. Todhunter and Riordan noted in their letter that a number of questions exist about the environmental implications of genetically engineered organisms and that many of these questions affect the missions of

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[114] (continued)
Interagency Advisory Committee is to "advise ... the Secretary of the Department of Health and Human Services, the Assistant Secretary for Health, and the Director, National Institutes of Health, on the coordination of those aspects of all Federal programs and activities relating to recombinant DNA research. The Committee provides for communication and exchange of information necessary to maintain adequate coordination of such programs and activities. The Committee is responsible for facilitating compliance with a uniform set of guidelines in the conduct of this research in the public and private sectors and, where warranted, to suggest administrative or legislative proposals." 48 Fed. Reg. 24577 (1983). The Committee has representatives from over 17 federal agencies. Id. The Interagency Advisory Committee is discussed briefly in the OTA Report, supra note 2, at 214.

[115] Dr. Talbot related that the Committee met eleven times between 1976 and 1980. See response of Dr. Talbot to supplemental written questions in Appendix C to the hearing transcript. The Committee apparently has not met since 1980, however.

[116] See Appendix C to this report.

both agencies. "Because of the interrelationships among agencies," they stated "we think there would be merit in broadening the scope of the risk assessment work and to make it into a more collaborative venture."[119] Drs. Todhunter and Riordan were particularly supportive of interagency cooperation because of the uniqueness of the problems posed by the deliberate release of genetically engineered organisms. As they related:

> The EPA is charged by Congress to protect public health and our environment through a number of different statutes. We are concerned with contamination of water, drinking water and air, with solid waste and waste sites, and with pesticides and toxic substances. There is an impression that some of the commercial activities in the recombinant DNA field are being developed without the benefit of criteria for judging general population and environmental safety of the cellular products and especially the life forms themselves. We at EPA are charged with developing responsible public policy. Currently, it would seem prudent to investigate "potential" hazards associated with recombinant DNA developments for which we lack sufficient information to substantiate or refute our concerns. Therefore, we hope that further risk assessment research of an expanded nature will be undertaken; we are eager to work with you and other agencies in developing a strategy ...[120]

The needed cooperation between the various agencies can best occur within the context of an interagency group, as has been proposed herein.

In recommending the creation of an interagency task force, the staff has recommended that the NIH RAC cease its practice of evaluating and approving proposals for deliberate releases from commercial biotechnology firms. As the discussion earlier in this report indicated, biotechnology companies are not legally required to seek RAC approval for releases. Although the RAC may be empowered to review these proposals if commercial firms voluntarily come before them, the RAC's decision is not legally binding on the companies. Indeed, the effect of a decision by the RAC on a proposal for a deliberate release is uncertain. The RAC certainly cannot prohibit a commercial company from proceeding with a release, and thus, its denial of a request actually amounts only to a warning of sorts to the company.[121] Conversely, RAC

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[117] *See* Appendix C to this report.
[118] *See* Appendix C to this report.
[119] *Id.*
[120] *See* appendix C to this report.

approval of a release provides no legally effective guarantee that the release is safe and complies with any federally established criteria. Such ambiguity is not conducive to rational and consistent regulation of deliberate releases, and this situation should be remedied by a cessation of RAC involvement with commercial biotechnology companies with regard to deliberate releases. The RAC should restrict its review to those activities over which it has clear legal jurisdiction -- i.e., NIH-funded research.

Additionally, it is recommended that the NIH RAC and the USDA RAC revise their respective memberships to include individuals who are specifically trained in ecology and the environmental sciences. There will be some applications for deliberate release, such as those resulting from NIH and USDA sponsored research, that each RAC will continue to review. In order to ensure that the environmental issues raised by these proposals received adequate consideration, each RAC must have individuals among its membership who are conversant in those issues. Currently, neither RAC contains an ecologist, and this deficiency must be corrected if each RAC intends to continue to review proposals for deliberate releases. This is not to say that the simple addition of an ecologist will make each RAC fully capable of evaluating release requests. Indeed, each RAC should include several individuals so trained.

Related to this consideration, there are are also concerns about the role of USDA in overseeing deliberate releases. As this report has related, USDA, through its RAC, is already involved to some extent in reviewing proposals for deliberate releases. As this report also has discussed, however, USDA may potentially have the authority to play a more extensive role in the regulation of this technology. It is neither desirable nor in the best interests of the environment or industry to have such uncertainty surrounding USDA's jurisdiction. This is especially so given the position taken by USDA regarding the extent to which it intends to be involved in overseeing releases. In order to clarify USDA's jurisdictional status, the staff has recommended that the Government Accounting Office (GAO) review USDA's activities in this area and evaluate the extent of its authority to regulate deliberate releases under all relevant statutes, regulations, and executive orders. Such a review and evaluation would facilitate the needed coordinated oversight of deliberate releases and expedite the work of the interagency task force.

While the NIH and USDA may, along with EPA, continue to be involved in reviewing proposals for deliberate releases, no deliberate release should be permitted by any federal agency until

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[121] This may provide a basis for tort liability, however. (Karny testimony p.47 in the hearing transcript).

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it has been reviewed by the interagency panel, and we have recommended such. Because of the diverse nature of the types of genetically engineered organisms that will likely be produced and the uses to which they will be put, each agency may well have jurisdiction over various releases. A principal function of the interagency task force is to minimize the effect of the balkanization of authority that exists within the government and to ensure that the environmental implications of each release are adequately evaluated according to a uniform set of guidelines. Before an agency makes a final determination regarding the safety of a given release, it should be reviewed by the interagency task force. Review and recommendation by the panel will not only provide consistency in the evaluative process; it will also ensure that the necessary expertise in both environmental questions and genetic engineering matters is employed in reviewing a proposal. Each agency will utilize the guidelines for releases developed by the interagency panel, and because each agency will participate in the panel, each will have ample opportunity to state its position on the proposed release.

Finally, it is recommended that EPA proceed with its effort to regulate deliberate releases under TSCA. However, as this report has noted, questions exist concerning whether TSCA actually empowers EPA to regulate recombinant DNA technology, and it is not unlikely that EPA's authority may be challenged in court. Based on EPA's conclusion that it does have such authority under TSCA, the staff feels that no additional legislation or clarifying amendments to TSCA are necessary at this time.

The recommendation here is not to be taken as an endorsement of EPA action under TSCA. As noted previously, EPA has yet to articulate either the scope or details of its regulatory effort. Moreover, EPA has experienced difficultly in implementing TSCA. EPA's current manpower shortages and proposed budgetary reductions imply that the agency's effort to regulate genetically engineered substances may not be overly successful either.

Given the uncertainty of EPA's actions, then, and the questions surrounding EPA's ability to regulate, the staff cannot conclude that EPA's effort will be either adequate or inadequate. It is not clear that sufficient environmental safeguards will exist even once the EPA has issued its regulations; that will depend on the regulations themselves. It is also unclear whether the burdens the regulations will impose on this nascent industry will be reasonable ones.

The principal concern is that adequate attention be given to the environmental implications of deliberate releases by a qualified body with minimum regulatory burden. The staff believes that the interagency task force recommended herein will meet that concern, and views EPA's effort to regulate under TSCA as supplemental to the activity of the interagency group.

This view is reflected in the recommendation that consideration be given to instituting the interagency task force as a permanent oversight entity even after EPA has issued its regulations. In the short run, the interagency task force would work to develop a functional procedure for evaluating environmental risks and also aid EPA in the development of its regulations under TSCA. In the long run, the task force would be a vital mechanism for reviewing proposals for deliberate releases by using information gathered by EPA under its TSCA regulations, and by other agencies, and considering it in an assessment of the potential risks posed by a new organism.[122] Essentially, EPA would gather the needed information through its regulations, and the interagency group would use this information in determining the existence or absence of an environmental risk. The work of the interagency task force and EPA's regulatory effort under TSCA would thus go hand-in-hand to ensure that environmental risks are adequately evaluated and any potential dangers minimized to the greatest extent possible. Minimizing these dangers should be the basic goal of any agency involved in oversight of biotechnology.

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[122] As noted previously, other statutes may also provide EPA with authority to regulate deliberate releases. Any information gathered under these statutes would also be utilized by the interagency task force.

APPENDIX A

Plant and Soil 48, 143-152 (1977) Ms. 3200

REASSOCIATION OF A MODIFIED MYCORRHIZA WITH THE HOST PLANT ROOTS (*PINUS RADIATA*) AND THE TRANSFER OF ACETYLENE REDUCTION ACTIVITY

by K. L. GILES and H. C. M. WHITEHEAD

Plant Physiology Division, D.S.I.R.,
Private Bag, Palmerston North, New Zealand

SUMMARY

Experiments were carried out to assess the potential for reassociation of modified strains of the mycorrhizal fungus *Rhizopogon* sp., capable of acetylene reduction activity *in vitro*, with the roots of its host plant (*Pinus radiata*). Reassociation was effected and acetylene reduction assays indicated that nitrogenase activity was present in the reassociated whole plants. Those host plants symbiotic with the modified strains had higher levels of nitrogen than those associated with the wild type fungus under nitrogen deprived conditions. Uptake of phosphate was unimpaired in the modified mycorrhiza. Electron microscopy showed that hyphae of the modified strains, as well as lying in the intercellular spaces, were often found within the cells of the root cortex. This was in contrast with the wild type strains where no such intracellular growth was found. One strain was found to be pathogenic to seedlings of *Pinus radiata*.

INTRODUCTION

Following the recently reported successful transfer of acetylene reduction activity to a mycorrhizal fungus (*Rhizopogon* sp.)[1, 2], a fungus normally mycorrhizal with the roots of *Pinus* seedlings, it has become of obvious interest to ascertain the nature of, and the factors affecting, the reassociation of this modified strain with seedling roots. The modified strains were produced by the induced uptake of whole cells of *Azotobacter vinelandii* into spheroplasts of the mycorrhizal fungus. The spheroplasts were produced by enzymic digestion of the fungal cell walls. The clone of Rhizopogon used in the initial experiments was isolated from the roots of *Pinus radiata*

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by Forest Research Institute, Rotorua, and *Pinus radiata* seedlings were therefore chosen for use in the reassociation experiments.

The nitrogen and phosphorus levels are of prime importance during both mycorrhizal reassociation and later symbiosis[4 5]. High levels of these elements tend to depress the level of fungal association with the host roots. The activity of the modified strains, capable of acetylene reduction and therefore presumably nitrogen fixation, could have been a barrier to the reassociation because of the levels of fixed nitrogen. Coupled with this, since the whole bacterial genome was introduced into the fungus, other factors present in the modified strains may have been inhibitory to any reassociation with the host plant.

In the legume nodule system, light increases the rate of nitrogen fixation because of the increased levels of photosynthate available as an energy source. Tests were carried out to ascertain whether similar effects occurred in this association.

MATERIALS AND METHODS

Five modified strains of Rhizopogon were grown on nitrogen-deficient Hagem's medium[1]. Pine seeds, obtained from Forest Research Institute, Rotorua, were surface sterilised in 1% hypochlorite solution and then planted into sterile pumice and vermiculite (1–1). Seedlings were grown under sterile conditions with a full Hoagland's medium applied every two days to the medium for a period of 3 months. When 3 months old 200 seedlings, selected for their similarity in size were potted up into sterile pumice and vermiculite and were divided using a double blind technique into eight lots of 25. Two batches of 25 (A, B) were inoculated with cultures of the modified Rhizopogon strains as follows. Cultures of five different strains isolated in the original series of experiments were diced and the pieces placed in close association with the seedling roots and mixed throughout the potting medium. Five seedlings were inoculated with each of the five strains. A second two batches of 25 plants each (C, D) were inoculated with the wild type cultures of Rhizopogon which had been grown on full Hagem's medium. A further 50 seedlings were potted up in the sterile potting mix and were non-inoculated (E, F). Seedling trees A-E received modified Hoagland's nutrient containing 10% of the combined phosphorus (1.5 ppm) and no combined nitrogen or molybdic acid; calcium chloride replacing calcium nitrate, and potassium chloride replacing potassium nitrate in the solution. Trees in groups B and D were given 20 ml of 0.005 g/litre molybdic acid every 2 days. Seedlings in group F received full Hoagland's medium and acted as a non-inoculated, non-deprived control. The seedling trees were maintained in a greenhouse under controlled temperatures of 25°C daytime, 15°C night for a period of 3 months.

After this period seedlings were unpotted and visual signs of mycorrhizal association were looked for. Plants were tested for acetylene reduction activity using a Perkin-Elmer gas chromatograph with a 25 cm column containing 0.35 g of Poropak T. The column was maintained at 75°C, and nitrogen and oxygen pressures ran at 10 lb/in^{-2} and hydrogen at 20 lb/in^{-2}. A 10% atmospheric acetylene concentration was used in the assay bottles and 0.2 ml samples were taken at intervals over a period of 6 hours. Seedlings were contained in 200 ml bottles fitted with serum tops and were maintained at 25°C in a water bath.

Because of the low levels of acetylene reduction detected stringent controls were necessary to maintain reproducibility. The background level of ethylene in the acetylene used was measured regularly and allowance made for it in measuring the amounts of ethylene produced. New serum caps were used and this helped to keep the loss of acetylene from the system to a minimum over the six hour assay period. Ethylene production was calculated from the ethylene to acetylene ratio. No ethylene production by the tissue was detected in the absence of acetylene.

Pieces of the reassociated root systems were taken for electron microscopy, fixed in Karnovsky's fixative (3% glutaraldehyde, 2% formaldehyde in 0.05 *M* phosphate buffer at pH 6.8), post-fixed in osmium and stained with alcoholic uranyl acetate and lead citrate.

Selections of the infected root were placed on nitrogen deficient Hagem's medium to re-isolate the mycorrhizal fungus. The activity and ultrastructure of these isolated modified strains of Rhizopogon were studied as described above.

Total nitrogen was determined by a modified Kjeldahl method. Phosphorous was determined using a colorimetric technique[3] after wet ashing samples with perchloric acid.

RESULTS

Examination of the roots of groups E and F showed no signs of mycorrhizal association after a 3 month period deprived of nitrogen. Neither of these groups had been inoculated. Group F which had been receiving full Hoagland's medium during the 3 month period showed no signs of chlorosis or browning, but group E trees were much smaller in size and the needles were severely yellowed. Upon receiving full Hoagland's nutrients after the three month deprivation group E recovered fully and none died. Twenty-one plants in group C and nineteen in group D showed mycorrhizal reassociation. These groups had received inoculants of the wild-type mycorrhiza and had received nitrogen deficient, phosphorus-low Hoagland's during the course of the 3-month period. The plants showed a white fluffy mantle around many doubly bifurcated rootlets and a Hartig net, indicating mycorrhizal reassociation (Fig. 2A). Group D which had received added molybdenum during the course of the nutrient deprivation showed no greater signs of reassociation than did C. (121 ± 12 mycorrhizal rootlets per plant for D, compared with 130 ± 15 for C)*. Groups A and B which had been inoculated with the modified strains of Rhizopogon also showed doubly bifurcated roots, though the mantle was much less prominent and in some cases almost lacking (Fig. 2b). Fourteen plants in Group A and twelve in Group B which had received molybdenum supplement during the course of the deprivation did have a higher rate of mycorrhizal association than A (158 ± 17 mycorrhizal rootlets per plant for B, compared with 115 ± 10 for A). Seedlings inoculated with strain 1 in both groups A and B died, seemingly because the fungus was not

TABLE 1

Acetylene reduction activity of modified fungal strains 1–5 in plant groups A and B**

Group	Strain	Acetylene reduction activity nmoles C_2H_4/ g DW mycorrhizal rootlet/hour	mg/g nitrogen dry weight of roots and shoots
A	1	—	
	2	2.64	
	3	3.27	
	4	2.51	
	5	2.83	940 ± 25
B	1	—	
	2	3.38	
	3	4.20	
	4	2.45	
	5	3.67	
C, D		0	820 ± 39
E		0	808 ± 45
F		0	1040 ± 12
B_2 reisolated		0.77 mg DW mycelium	

* Plants had 0.5 g to 2 g DW of mycorrhizal rootlet each. Strain 1 killed the seedlings it became associated with and no assays could be performed. All other figures represent the average for the group of five. Strain B_2 was reisolated from the seedling roots and its activity is expressed per mg dry weight of mycelium. Nitrogen content is based on an assay of 50 plants in groups A-D and on 25 plants in groups E and F. Variability indicates the standard deviation. Acetylene reduction assays were performed in 15 ml vials with a background sensitivity of 0.5 nmoles h^{-1} g^{-1}.

** Variability indicates the standard deviation.

controlled in the root cortex but grew throughout all parts of the plant, and even appeared around the leaf bases as small tufts of hyphae.

Acetylene reduction activity.

All seedlings were assayed for acetylene reduction activity. Seedlings inoculated with strains 2 to 5 in both groups A and B showed detectable acetylene reduction activity in 60% of the reassociated trees. The activity in B was about 20-25% higher than activity in A. No activity was recorded in seedlings lacking well formed mycorrhizal rootlets. No activity was recorded in Groups C–F (Table 1). Table 2 shows the effects on acetylene reduction activity using mycorrhizal rootlets, small pieces of root attached to the rootlets, or the whole seedling in the assay vials. The mycorrhizal rootlets had low but detectable activity. Rootlets still attached to pieces of the root, about 2.5–4 cm long, gave a much higher figure expressed as dry weight of mycorrhizal rootlets. The activity was much enhanced when the mycorrhizal rootlets were left attached to the intact plant. The levels varied depending whether the experiment was performed in the light or the dark. In the light

TABLE 2

The effects of using mycorrhizal rootlets, pieces of root with rootlets, and whole plants in the acetylene reduction assay*

	nmoles C_2H_4/g DW mycorrhizal rootlets/h	
	1	2
Rootlets	0.773	0.57
Roots plus rootlets	3.18	2.83
Whole plant (8–10 cm)		
Light	45.6	38.2
Dark	1.2	1.65

* Results are shown for two plants, 1 and 2, and the whole plants were tested both in continuous light and dark. The light-dark effect was reversible (see Fig. 1). Upon removal of the mycorrhizal rootlets the activity fell to below the sensitivity of the detection method used, indicating that the nitrogenase activity was associated with them.

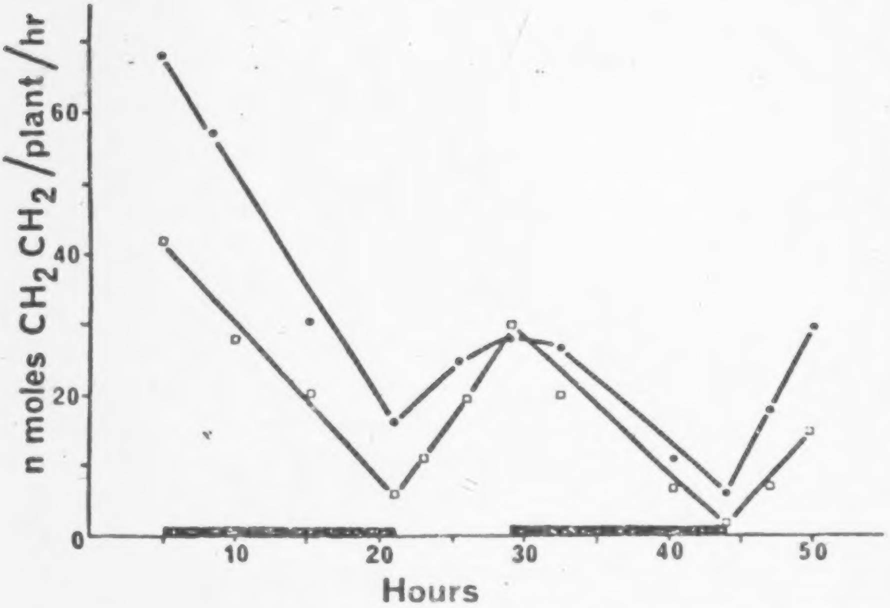

Fig. 1. Acetylene reduction activity of whole plants associated with the modified mycorrhiza in fluctuating light/dark cycles. (16-h dark periods). Trees were maintained at 25°C during the experiment and a 10% acetylene atmosphere was used throughout.

whole plants had an activity of up to 45.6 nmoles of acetylene g^{-1} mycorrhizal rootlets h^{-1}, whereas in the dark only about 1/50 of this activity was recorded. This effect was reversible in light and dark over a period of at least 60 hours (Fig. 1).

In order to ascertain that there was not a trivial explanation for the acetylene reduction activity recorded in association with the plant a series of controls were run in parallel with all the determinations. As well as seedlings associated with the modified fungal strains seedlings reassociated with the wild-type fungus, similar to those seedlings in Group C, were also assayed. Seedlings unassociated with mycorrhizae were assayed for ethylene production both with and without acetylene in the assay bottle. Heat killed seedlings, treated at 110°C for six hours were also used as a control. Seedlings reassociated with the modified strains were assayed after removal of the mycorrhizal rootlets in order to determine that this was the site primarily responsible for the nitrogenase activity. All these controls gave no ethylene production above the sensitivity limits, calculated for whole plants in 200 ml flasks, being about 7 nmole.$h^{-1}.g^{-1}$. These readings were considered as background and subtracted from the assays of seedlings reassociated with modified fungal strains.

The comparison of analyses of shoots and roots of control and inoculated plants showed that controls receiving full Hoagland's had a higher nitrogen content than trees under nitrogen deficient conditions, whether they were inoculated with wild type or modified strains of Rhizopogon. However, those trees under nitrogen deficient conditions inoculated with a modified Rhizopogon capable of acetylene reduction had an intermediate level of nitrogen in both roots and shoots, compared with those inoculated solely with the wild type Rhizopogon indicating that some incorporation of fixed nitrogen may have occurred (Table 1). Mycorrhizal rootlets were included in total nitrogen measurements so that some of the recorded nitrogen will be fungal in origin. Phosphorus levels were the same in all treatments, indicating that phosphate uptake by the modified strains was probably not impaired (Table 3).

TABLE 3

Analysis of total phosphorus*

Groups		Total P µg/g DW
A,B	Shoots	585
	Roots	515
C, D	Shoots	575
	Roots	519
E, F	Shoots	627
	Roots	601

* Fifty plants in each of the treatments (AB-modified mycorrhiza, CD-wild type mycorrhiza and EF-no mycorrhiza, non deprived control) were dried, powdered and mixed and samples assayed. There was no significant difference in phosphorus content between plants associated with modified or wild type mycorrhizae, though both had a lower phosphorus content than non-deprived controls. This indicates that phosphorus uptake by the modified and wild type fungus was comparable.

Electron microscopy revealed the presence of fungal hyphae in the intercellular spaces of the cortex of seedling roots (Fig. 2C). Hyphae were also present closely adhering to the outside walls of the epidermal cells of the root. These fungal hyphae seemed identical ultrastructurally with the hyphae examined from *in vitro* cultures. When comparing the ultrastructure of the roots inoculated with modified and non-modified strains it was found that hyphae of the modified strains quite frequently intruded into the intracellular spaces of the cortical cells of the root. At no time was this noticed with the control Rhizopogon strains. The cells into which the modified strain had entered appeared dead and were empty of cytoplasm. Whether this

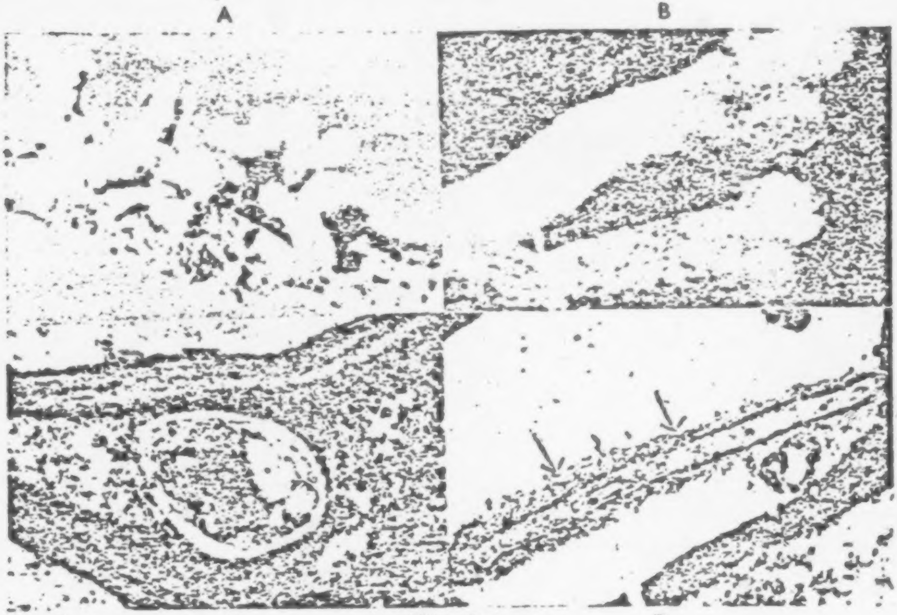

Fig. 2

A. Mycorrhizal rootlets on *Pinus radiata* associated with wild type Rhizopogon. Doubly bifurcating rootlets are present three months after reassociation and a mantle is present as a white 'fluff' on the root surface. × 7.

B. Mycorrhizal rootlet on *Pinus radiata* associated with the modified strain of Rhizopogon (strain 2). Although the rootlet has become bifurcated only a very sparse mantle is present. × 21.

C. Cross-section of the Hartig net of modified strain 2 of Rhizopogon in the intercellular space within the root cortex of *P. radiata* × 15,500

D. Hypha of the modified strain 2 of Rhizopogon in a dead cortical cell of *P. radiata*. Arrows indicate poly-β- hydroxy - butyrate globule characteristic of the strain. × 1,800 was due to the presence of the fungus, or whether the fungus had merely entered the dead cells is not known. The hyphae within these dead cells were identical with the *in vitro* cultures containing small globules of polyhydroxybutyrate and the spherical bodies seen in cultures of the modified strain[1 2] (Fig. 2D). Hyphae reisolated from the reassociated roots retained their normal morphology and acetylene reduction activity (Table 1).

DISCUSSION

The reassociation of the modified strains of Rhizopogon with the roots of *Pinus radiata* is encouraging. The fact that batch B inoculated with modified Rhizopogon receiving molybdenum, a necessary co-factor in nitrogen fixation, should produce better and more numerous mycorrhizal rootlets is also interesting, since molybdenum had no effect with the control mycorrhiza.

In batch A and B acetylene reduction activity was detectable in 60% of the reassociated plants. The reasons for the lack of activity in some reassociated plants is not clear, but may be associated with sectoring of the *in vitro* cultures. The high levels recorded during the light period are presumably related to the photosynthate transported and being available to the mycorrhizal fungus as an energy source. The lower number of seedlings reassociating with the modified strains, (Groups A and B), where only 26 out of 50 reformed the symbiosis, compared with 40 out of 50 when inoculated with the wild-type strain, is presumably due to some alteration in the physiology of the fungus due to bacterial uptake, or to the manipulations involved in that process.

The most disturbing feature of these findings was the pathogenicity of strain 1 of the modified fungus and the intracellular occurrence of the fungal hyphae. Whether this intracellular growth indicates a sub-pathogenic infection is not known. The ten trees which had been treated with strain 1, the strain which had the highest acetylene reduction activity *in vitro*, were taken over by hyphae which seemed to infiltrate all parts of the plant. The seedlings, were about 10 cm in height and all five trees in both groups A and B died. Strain 1 of the fungus and the trees inoculated with it were autoclaved and sterilely destroyed. The other four strains of fungus superficially had no pathological effects on the trees, and it is only the intracellular habit which prompts caution. It should be noted that Rhizopogon is normally mycorrhizal with the roots of seedlings of many species of Pinus and it may be that more extreme pathogenic symptoms may be expressed on the roots of other species than *P. radiata.* This possibility is presently being tested and until more information is available all strains are being grown only under restricted sterile conditions and under no circumstances being released for field trials.

The finding of acetylene reduction activity in plant roots reassociated with these modified strains, and the increased nitrogen content of reassociated plants, is a hopeful sign that this form of transfer of nitrogen fixation, using genetically modified mycorrhizas, may be successful. The sub-pathogenic nature of the reassociation makes it necessary to scan a wide range of species for their reaction to these modified strains. It also raises the possibility that another fungal host might be preferable to Rhizopogon in such transfers as this, or that a different free-living nitrogen-fixing bacteria could be introduced. In either case these results support the contention that the genetic engineering or modification of rhizosphere organisms, capable of a symbiotic relationship with higher plants might be a worthwhile field to examine. These findings, both of the transfer of acetylene reduction activity into a eucaryote cell and the reassociation of the modified fungus with the host plant, represent first reports of the potential transfer of nitrogen fixing ability and much more work is necessary to ensure such systems are both biologically safe and effective before anything can be said of their potential agronomic role.

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The transfer of nitrogen fixing ability to a eukaryote cell

K. L. Giles and H. C. M. Whitehead

Plant Physiology Division, D.S.I.R., Palmerston North, New Zealand

Abstract

Cells of *Azotobacter vinelandii* have been introduced into spheroplasts of the mycorrhizal fungus *Rhizopogon*. Polyethylene glycol (PEG) was used to induce uptake. Five strains of the fungus were isolated, from 7.5×10^6 spheroplasts, capable of both growth on nitrogen deficient culture media, and the reduction of acetylene in the acetylene reduction assay for nitrogenase activity. Poly-β-hydroxybutyric acid (PHB), a storage product in the cysts of *Azotobacter*, was detected in the modified fungal strains but was absent in the control fungus. No intact bacterial cells were identified within the hyphae, suggesting that some degree of digestion had occurred within the hyphae. Morphological changes in the mitochondria of the modified fungus were found and these are discussed with reference to possible oxygen-protection mechanisms. Acetylene reduction activity has been detected in the fungal cultures for a period of seven months, during which time acetylene reduction activity decreased to a low level. This is the first report of the transfer of nitrogen fixation into a eukaryote cell by transgenosis, and possible extensions of this type of study are discussed.

Introduction

There is considerable interest in the possibility of transferring the ability to fix atmospheric nitrogen to agronomically important species, other than legumes (Cocking and Davey, 1973; Postgate, 1974a). Recently there have been reports of systems, involving both *Azotobacter* (Carlson and Chaleff, 1974) and *Rhizobium* (Scowcroft and Gibson, 1975; Child , 1975), being established in callus tissue systems, but there has been no demonstration of the transfer of the genetic information responsible into whole organisms.

Although the introduction of free-living nitrogen-fixing bacteria into plant protoplasts might represent a direct approach to such a transfer of information, current techniques only allow the culture and regeneration of protoplasts derived from a relatively few species of higher plants. The protoplasts, or spheroplasts (Villanueva and Garcia Acha, 1971), of fungi, however, will regenerate cell walls and many readily revert to a normal mycelial growth form (Peberdy, 1972). Fungi also have the advantage that many of those living in the soil form mycorrhizal associations with the roots of higher plants, particularly tree species. Most forest trees develop ecto-mycorrhiza (Trappe, 1962) which aid in the absorption of inorganic and organic nutrients (Melin, 1958). If it were possible to introduce nitrogen fixation into this class of fungus it is probable that some proportion of the fixed nitrogen would become available to the host plant, giving it some independence from any nitrogen deficiency in the soil or supplementing the nitrogen which is available. This paper describes the first step in the formation of such a nitrogen-fixing symbiosis, the introduction of nitrogen fixation into a mycorrhizal fungus.

Materials and methods

A culture of *Rhizopogon* sp. supplied by the Forestry Research Institute, Rotorua, was grown in Petri dishes at 25°C on Hagem's medium (9.4 mM, NH_4Cl; 3.4 mM, KH_2PO_4; 2 mM, $MgSO_4.7H_2O$; 1 ml/1 of 1% $FeCl_3$; 5 g glucose/1; 5 g malt extract/1; solidified with 1.5% agar), and subcultured every 10 days. The fungus was shown to be mycorrhizal with the roots of *Pinus radiata* under glasshouse conditions by both the Forestry Research Institute, Rotorua, and this laboratory.

Fungal spheroplasts were prepared from 2- to 3-day-old cultures of *Rhizopogon* using an enzyme preparation from *Trichoderma viride* as described by de Vries and Wessels (1972). The enzyme preparation was used at a concentration of 2 mg protein/ml. $MgSO_4$ (0.5 M) was the osmotic stabilizer dissolved in 0.05 M Na-maleate buffer, pH 5.8. Small pieces of mycelium were washed in this solution and 50–100 μg dry weight were incubated in 100 μl of the enzyme-supplemented osmoticum at room temperature. Eight to 10 h incubation was sufficient to release most of the spheroplasts. Yields were variable between 2×10^4 per ml and 10^6 per ml. The spheroplasts were isolated from the mycelial debris by flotation of the vacuolate spheroplasts.

A culture of *Azotobacter vinelandii* was grown on Ashby's medium (3.2 mM, K_2HPO_4; 2 mM, $MgSO_4.7H_2O$; 0.1 mM, $FeSO_4.7H_2O$; 0.1 mM, $MnSO_4.2H_2O$; 0.04 mM, Na_2MoO_4; 1.7 mM, NaCl; 5 mM, $CaCO_3$; 5 g, glucose; solidified with 1.2% agar per litre, pH 8). The bacteria were subcultured every 5 days to fresh medium.

Uptake of bacterial cells by the fungal spheroplasts was induced by the presence of 20% w/v polyethylene glycol (PEG) (MW 6000), as described by Bonnett and Erikssen (1974), for the uptake of chloroplasts in to higher plant protoplasts. Half a millilitre of a suspension of fungal spheroplasts in 0.5 M $MgSO_4$, at a concentration of 2.5×10^5/ml, were mixed with 0.5 ml of a suspension (2.5×10^6/ml) of vegetative cells of a 3-day-old culture of *Azotobacter*, also suspended in 0.5 M $MgSO_4$, and 1 ml of 40% w/v PEG was added. Ten min after mixing, the suspension was transferred to an 8 μm Millipore filter and washed free of PEG with 0.5 M $MgSO_4$-supplemented nitrogen-deficient Hagem's medium. Microscopic examination of the spheroplasts after washing indicated that most of the bacteria had been washed from the spheroplasts. Three h later, allowing time for uptake, any adhering bacteria still outside the spheroplasts were lysed with a 500 μg/ml lysozyme solution. The spheroplasts were visually unaffected by the lysozyme wash since most had by this time become osmotically insensitive.

After the lysozyme wash, the spheroplasts were resuspended in 0.5 ml of liquid Hagem's medium still supplemented with 0.5 M $MgSO_4$ and spread on the surface of nitrogen-deficient Hagem's medium solidified with 1.2% agar. In nitrogen-deficient Hagem's

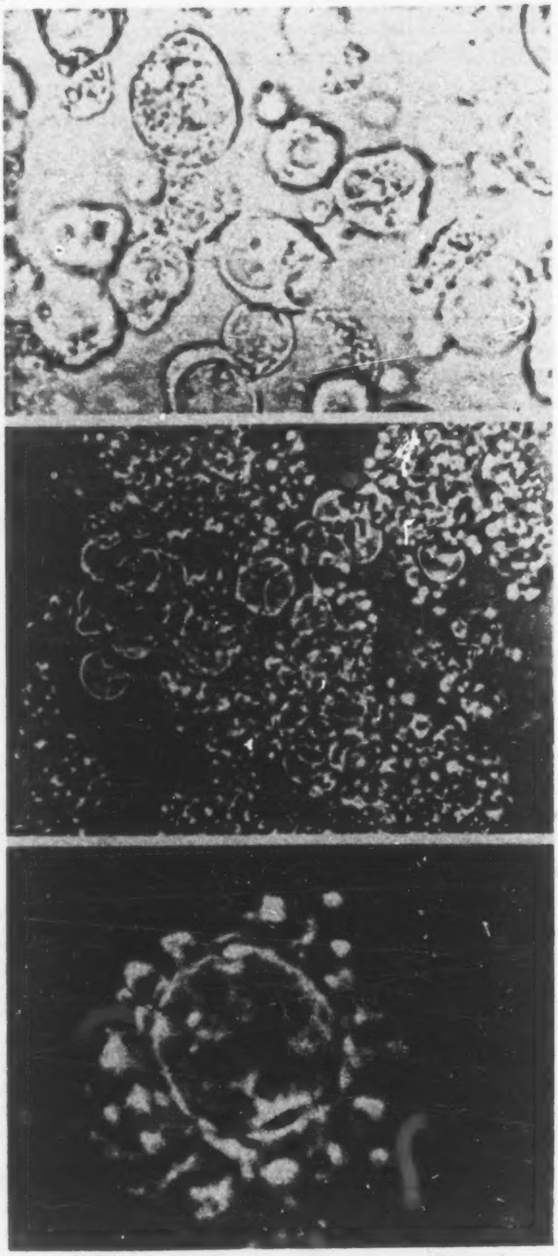
Figure 1 Uptake of *Azotobacter* cells by isolated fungal spheroplasts of *Rhizopogon* sp.

Figure 1a (top) Spheroplasts of *Rhizopogon* at the time of isolation suspended in 0.5 M $MgSO_4$. × 600.

Figure 1b (centre) Mixture of fungal spheroplasts and *Azotobacter* before addition of PEG. The fungal spheroplasts tend to stick together during cell wall regeneration but no fusion occurs. × 350.

Figure 1c (bottom) Fluorescence micrograph of fluorescein stained *Azotobacter* cells closely surrounding an unstained fungal spheroplast in the presence of PEG. The arrows indicate two fluorescing bodies within the spheroplast, indicating possible bacterial uptake. × 1,000.

Figures 1a, 1b and 1c



medium KCl replaced NH_4Cl and the malt extract was replaced by 0.5 mg/l nicotinic acid, 0.1 mg/l pyridoxine-HCl, and 0.1 mg/l thiamine-HCl. Benzylpenicillin at a concentration of 400 μg/ml was added to this medium. Previous tests had shown that this concentration lysed the strain of *Azotobacter* used without affecting the regeneration or reversion of the fungal spheroplasts.

The acetylene reduction assay for nitrogenase activity was performed using a Perkin Elmer gas chromatograph. A 30 x 0.15 cm column of Poropak T was used at an oven temperature of 75°C. Discs of agar containing the fungus were incubated at 25°C in an atmosphere of 90% air, 10% acetylene and 0.2 ml samples withdrawn to detect ethylene production. Ethylene production was linear over the 4 or 6 h period of assay. Controls were run with either the fungus and no acetylene, or acetylene and no fungus. Ethylene controls were also run. Poly-β-hydroxybutyric was identified using the method of Law and Slepecky (1961).

Portions of the mycelium of both the control and modified *Rhizopogon* were fixed for electron microscopy in 3% glutaraldehyde and 2% formaldehyde in phosphate buffer, pH 6.8, at room temperature postfixed in 2% OsO_4 in 0.1 M phosphate buffer for 3 h. They were embedded in Durcupan resin and stained with uranyl nitrate. The control and modified strains for electron microscopy were grown under the same conditions, except that the modified strain was on nitrogen-deficient medium.

Attempts were made to locate the bacterial DNA using Auramine O in a fluorescent Feulgen method (Ruch and Bosshard, 1966).

Results

The fungal spheroplasts varied widely in size, from 20–80 μm and most of them were vacuolate (Figure 1a). PEG treatment caused an immediate clustering of bacterial cells around the spheroplasts, though it seemed to cause little fusion between spheroplasts (Figures 1b and c). There was a tendency for the fungal protoplasts to adhere, though no fusion seemed to occur during cell wall regeneration, both with and without PEG present.

Within 22 h of plating on Hagem's medium containing combined nitrogen, 70–80% of the unmodified fungal spheroplasts, i.e. those not exposed to bacteria, had regenerated a cell wall, shown two to three yeast-like buds and finally reverted to a normal mycelial hypha. On nitrogen-deficient medium, 10–15% of the unmodified spheroplasts regenerated cell walls and some of these showed occasional budding, but none of the 5 x 10^6 spheroplasts plated out reverted to mycelial growth. The PEG treatment given to induce bacterial uptake had no effect on the level of spheroplast reversion on Hagem's medium containing nitrogen. Slightly higher rates of regeneration and reversion could be obtained using liquid culture medium, 85–95% reversion, but this method greatly complicated the isolation of individual colonies for subculturing.

Out of 7.5 x 10^6 *Azotobacter*-treated fungal spheroplasts that were plated on to nitrogen-deficient medium, five colonies were isolated that were capable of reversion and growth. These colonies were isolated during the course of several experiments indicating the repeatability of the system. The control fungus on nitrogen-containing medium grew as a thick fluffy mat mainly on the surface of the agar (Figure 2). The modified fungal mycelium had a similar growth form in the presence of nitrogen, but on deficient media grew as a sparse mycelium of hyphae under the agar surface. The mycelium of

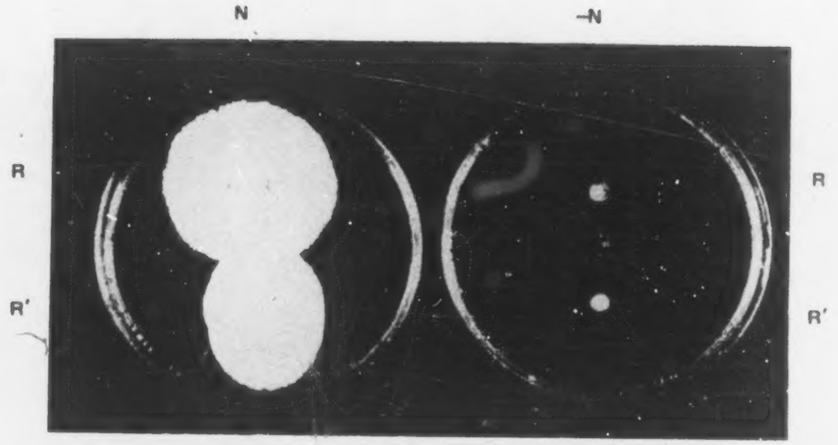


Figure 2 Growth forms of *Rhizopogon* strains on +N and −N media. Control *Rhizopogon* (R) growing as a thick mat on +N Hagem's medium but failing to grown on −N medium. Modified nitrogen-fixing *Rhizopogon* (R') growing in a similar manner as the control fungus on +N medium, but as a sparse mycelium under the agar surface on −N medium. All the colonies are 3 weeks old from the same sized inoculum in 9 cm Petri dishes.

D

the control fungus on deficient medium hardly grew off the nitrogen-containing agar inoculum (Figure 2). Both with and without combined nitrogen the control fungus diffused a yellow pigment into the medium. This was absent or markedly reduced in the cultures of the modified fungus on nitrogen-deficient medium, presumably due to the altered metabolism of this strain.

Forty-eight h after the original plating out the very small modified colonies were transferred under a binocular microscope to fresh medium lacking penicillin, and 96 h later they were again washed with 500 μg/ml lysozyme solution. The cultures were then transferred to fresh nitrogen-deficient medium and subjected to rigorous contamination tests. Portions of the mycelium were used to inoculate liquid Ashby's medium, to favour the growth of any contaminating *Azotobacter*. Seventy-two h after inoculation, and incubation at 25°C, samples were taken and plated on to Ashby's medium solidified with 1.2% agar. No colonies of *Azotobacter* were found and no acetylene reduction was detected. Non-inoculated controls gave negative results, whereas *Azotobacter*-inoculated flasks yielded many colonies which were actively reducing acetylene after 48 h. Portions of the modified mycelium left in the liquid medium for up to 3 weeks also gave negative results. The control *Rhizopogon* mycelium yielded two contaminants, a Gram-positive rod and a Gram-positive coccus, neither of which were capable of reducing acetylene. These two contaminants were not found in the modified fungal cultures.

Because of the tendency of the modified fungal hyphae to grow through the agar, rather than on the surface, it proved impossible to weigh the hyphae. Acetylene reduction measurements were made using five standard discs (5 mm diameter) of the agar and mycelium in each assay vial. The fresh weight of hyphae per disc was estimated to be between 2 and 6 mg.

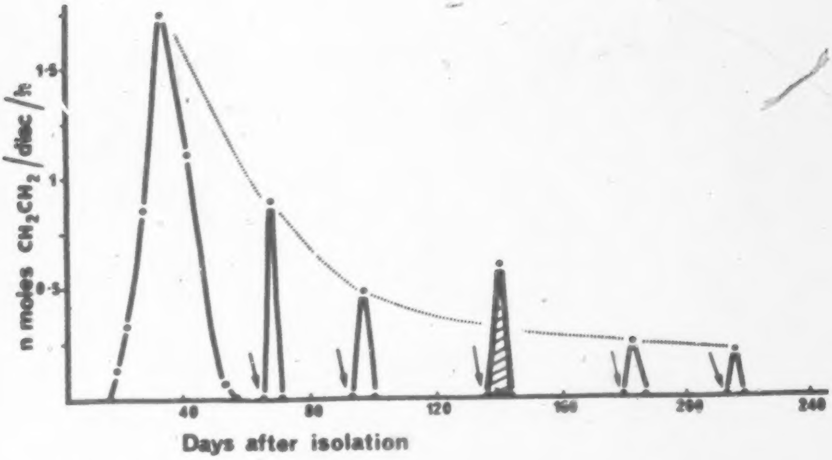


Figure 3 Acetylene reduction activity of one of the modified fungal strains. Arrows indicate transfer to fresh nitrogen-deficient medium. The shaded peak of activity was recorded from cultures derived from spheroplasts of the same strain. The gradual loss of activity is shown by the hatched line.

During the first 9 days in culture none of the five strains isolated reduced acetylene. Seventeen days after isolation acetylene was detected in four of the strains; the fifth did not show any activity until day 19. The level of activity increased until days 29–30 (Figure 3) and then decreased until, on day 55, there was no detectable acetylene reduction in any of the cultures. Upon transferring the cultures to fresh medium 61 days after isolation there was a second peak of activity lasting 6–7 days, peaking on day 3 in all of the cultures. This reappearance of acetylene reduction was repeated on days 93, 154, 182 and 212 when they were again subcultured to fresh medium (Figure 3).

After 138 days in culture, spheroplasts were again produced by enzymic digestion of the hyphal wall. The spheroplasts were plated on nitrogen-deficient medium and

Table 1 The effect of pH on acetylene reduction

pH	*Azotobacter vinelandii* nmoles CH_2CH_2/mg dry wt/h	Modified *Rhizopogon* nmoles CH_2CH_2/disc/h
8.5	240	–
8	878	–
7	134	0.2
6	93	0.56
4.7	55	1.4
4	13	0.9

Table 2 Effect of molybdenum on acetylene reduction activity in modified *Rhizopogon* hyphae

Conc of Na_2MoO_4 supplemented in Hagem's medium mg/l	Acetylene reduction activity nmoles CH_2CH_2/disc/h
0	2.42
1	3.6
2.5	5.9
3	5.7
5	5.6
10	5.9

Supplementing nitrogen deficient Hagem's medium with Na_2MoO_4 up to 2.5 mg/l increases the rate of acetylene reduction.

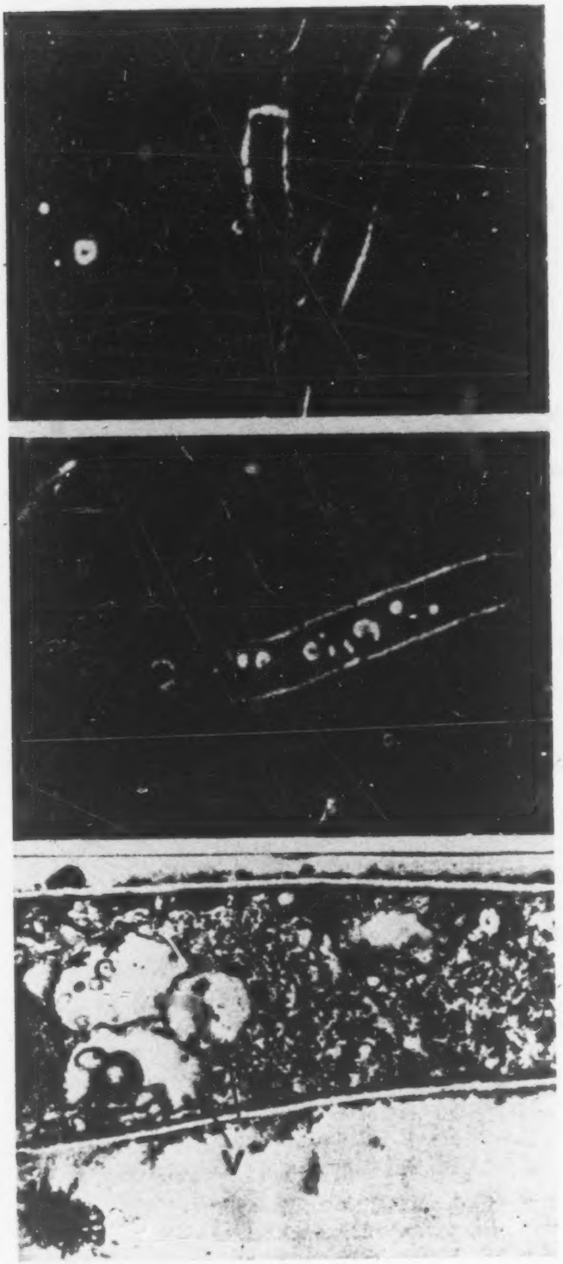
65–70% reverted to mycelial growth. Acetylene reduction was detected after 72 h and lasted for a further 6 days (Figure 3). Samples of the unmodified *Rhizopogon* mycelium at no time either reduced acetylene, or produced detectable ethylene, during the course of these experiments.

Azotobacter grown on Ashby's nitrogen-deficient medium at pH 8 had a peak of acetylene reduction activity 24–36 h after plating out, of 800–1,200 nmoles $CH_2 = CH_2$/mg dry weight/h. When plated on Hagem's nitrogen-deficient medium at pH 4.7, the peak of activity came only after 5 days and was only equivalent to 50–70 nmoles $CH_2 = CH_2$/mg dry weight/h. When moved to fresh medium 60 days after the initial transfer to Hagem's medium there was no reappearance of activity. These results are in contrast to the activity detected in the fungus, which appeared after 17 days of culture on Hagem's medium and was repeatable on transfer to fresh medium. The pH optimum for acetylene reduction by *Azotobacter* was pH 8, whereas in the fungus, optimum reduction occurred at pH 4, near the pH optimum for the growth of *Rhizopogon* (pH 3.8) (Table 1). Both of these findings support the contention that the acetylene reduction detected was not due to contaminating bacteria.

Supplementation of the nitrogen-deficient Hagem's medium with 2.5 mg/l Na_2MoO_4 increased the rate of fixation by the fungus two-fold, from 2.42 to 5.9 nmoles $CH_2 = CH_2$/disc/h on day 112. Higher concentrations did not appreciably raise this level, though lower ones were not as effective (Table 2).

Attempts to locate intact bacterial cells inside the fungal hypha have been unsuccessful. Protoplasts were made of the modified strains, lysed and plated on Ashby's medium, but no acetylene reduction or colonies of *Azotobacter* were detected. Optical microscopy revealed that the *Azotobacter*-treated strains had obvious inclusions within the hypha and these were lacking in the control mycelium (Figures 4a and b). It was at first thought that these were *Azotobacter* cells within the fungal symplast. Electron microscopy showed that these inclusions were deposits of an electron transparent storage deposit (Figures 4c and d). The control fungus had no such deposits within the hyphae. Similar deposits were also found in the cysts of *Azotobacter* and because

Figures 4a, 4b and 4c



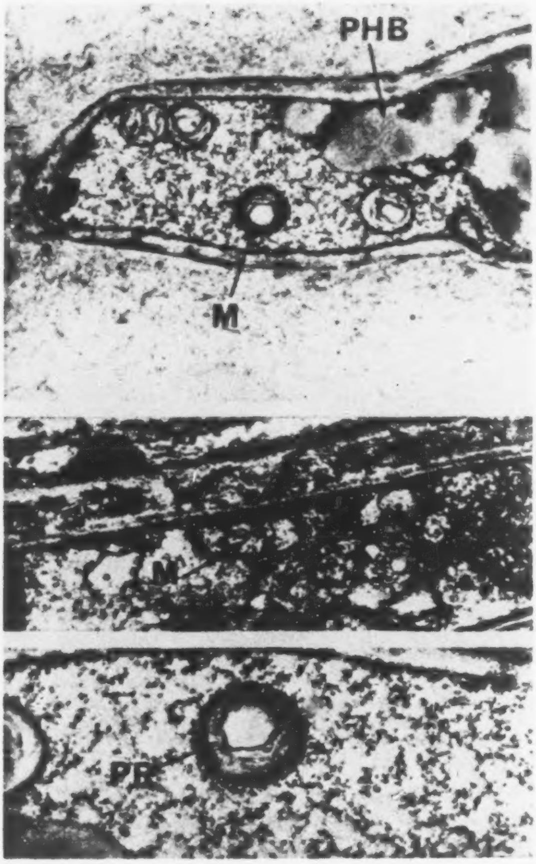


Figure 4 Structural changes associated with the transfer of nitrogen-fixing ability

Figure 4a (top, opposite) Hyphae of the control *Rhizopogon* lacking any obvious deposits of poly-β-hydroxybutyric acid. x 500.

Figure 4b (centre, opposite) Hyphae of the nitrogen-fixing strain of *Rhizopogon* showing intercellular deposits identified as poly-β-hydroxybutyric acid. x 500.

Figure 4c (bottom, opposite) Electron micrograph of the control *Rhizopogon* showing vacuoles (V), mitochondria (M). x 1,750.

Figure 4d (top) Electron micrograph of the modified nitrogen-fixing strain of *Rhizopogon* with mitochondria (M), poly-β-hydroxybutyric acid deposits (PHB). x 1,750.

Figure 4e (centre) Typical mitochondrion of the control *Rhizopogon*. x 3,600.

Figure 4f (bottom) Mitochondrion of nitrogen-fixing strain of *Rhizopogon* with surrounding polyribosomes (PR). x 3,600.

these have been identified as poly-β-hydroxybutyric acid (PHB) (Lemoigne and Girard, 1943), tests were carried out to determine whether this product was present in the modified fungal strains capable of acetylene reduction using the spectrophotometric method of Lemoigne and Girard (1943). It was found that PHB was present in the modified fungal hyphae, but that none was detectable in cultures of the control *Rhizopogon*.

The morphology of the mitochondria of the modified fungus was distinct from that of the control fungus. In the nitrogen-fixing strains the mitochondria were circular in section and were presumably spheres about 10 µm in diameter. They were usually surrounded by many polyribosomes and they seemed very constant in size and shape (Figure 4f). Mitochondria of the control fungus are much more irregular in shape and size (Figure 4e). Auramine O in the fluorescent Feulgen mode did not show any increase, or concentration of, possible bacterial DNA within the cytoplasm.

Discussion

With the introduction of the acetylene reduction technique for the assay of nitrogenase activity it has become clear that the natural mycorrhizal system cannot be regarded as a nitrogen-fixing association (Silvester and Bennett, 1973; Postgate, 1974b), and there is now no substantiated report of nitrogen fixation by any other eukaryote cell (Postgate, 1974a). Thus the introduction of nitrogenase activity into *Rhizopogon* cannot be regarded merely as gene induction, but represents information transfer, followed by transcription and translation of foreign nucleic acids, and the expression of foreign enzymes within the fungal cytoplasm. This is a remarkable series of events and constitutes transgenosis as defined by Doy, Gresshoff and Rolfe (1973).

The low number of colonies isolated capable of fixation and growth on deficient medium, five out of a total of 7.5×10^6 spheroplasts, may be a low estimate of the number of spheroplasts which actually took in *Azotobacter* cells. Many spheroplasts at the time of isolation lack nuclei, and these tended to be densely cytoplasmic and to be separated out during purification by flotation, but it is likely that some were carried up by the buoyancy of surrounding spheroplasts and were counted. However, even accepting some contamination, the very low percentage of the modified fungal spheroplasts reverting to mycelial growth must relate either to the number of bacterial cells taken up per spheroplast, or to their fate within the fungal cytoplasm. The fact that no intact bacterial cells were identified in the N_2-fixing strains, in many thin serial sections under the electron microscope, suggests that some degree of autolysis or digestion occurs within the fungus. It is possible that in many cases complete digestion of *Azotobacter* takes place and no stabilization of the bacterial genetic information occurs. In these cases the spheroplasts would fail to revert because of nitrogen deficiency.

Although intact bacterial cells were absent from the modified fungal hypha, the appearance of poly-β-hydroxybutyric acid (PHB) does confirm the presence of genetic information from *Azotobacter*. The control fungus never formed PHB in any of the growth conditions used and its occurrence in the modified strain is presumably due to a changed metabolism. Cells of the genus *Azotobacter* are known to develop specialized resting cysts and to deposit intracellular reserves of PHB (Stevenson and Socolofsky, 1967). These authors proposed that the accumulation of large amounts of PHB by

A. vinelandii reflects an unbalanced growth condition, which promotes cyst formation. They suggested that the cells assimilated exogenous carbon much faster than they could fix the molecular nitrogen necessary for conversion into nitrogenous cell components, and as a result the cells accumulated large amounts of non-nitrogenous material, PHB. The build-up of PHB within the fungal hypha indicates that not only the information for nitrogenase was transferred, but also that for the synthesis and accumulation of PHB. The PHB accumulation in the fungal hypha may reflect the inability of the fungus to fix nitrogen at a rate comparable to its rate of carbon assimilation, thus creating an unbalanced growth situation and triggering the PHB synthesis. An effect of this type could explain the intermittent nature of the activity in the modified fungus. The intermittent activity may, of course, also have been due to the removal of the fungus from a toxic environment, caused by the accumulation in the old media of extracellular toxins.

The extreme sensitivity of all nitrogenases to oxygen (Eady *et al.*, 1972) means that aerobic nitrogen-fixing organisms must have physiological mechanisms for protecting the functional enzyme. Heterocystous blue-green algae such as *Anabaena* and *Nostoc* have partly solved the problem by restricting nitrogenase activity to the heterocysts, which lack the oxygen-evolving photosystem 2 (Stewart, 1971). Fungi are fortunately free from the problems of photosynthetic oxygen evolution. *Azotobacter* was chosen for use in these experiments because it is reported to have two methods of nitrogenase protection. Firstly, the enzyme can change to a conformationally protected form which, though relatively inactive, is insensitive to oxygen, and secondly, respiration within the bacterial cell also acts to scavenge oxygen from the functioning enzyme (Postgate, 1974b). The growth of the modified fungal mycelium beneath the agar surface suggests a further way in which the oxygen tension might be lowered within the hypha.

The modified morphology of the mitochondria found within the fungal hyphae is of interest in relation to the possible respiratory protection of the *Azotobacter* nitrogenase. It is possible that the nitrogenase resides near, or in, the mitochondria, where it might be protected from excess oxygen and be close to a source of ATP. Fluorescent Feulgen technique failed to locate any extra concentration of DNA in the region of the mitochondrion.

The time-course for the expression of nitrogenase activity, as measured by acetylene reduction, is very similar to that described by Doy, Gresshoff and Rolfe (1973) for the expression of the Z gene of *Escherichia coli* in tomato cells. In the case of nitrogenase, no activity was detected until 17 days after treatment of the spheroplasts with *Azotobacter* cells, whereas in tomato cells 40 days elapsed before expression of the Z gene. The intermittent nature of the nitrogenase activity in modified strains of *Rhizopogon* closely parallels the behaviour of the β-galactosidase activity in transgenosed tomato cells which also showed repeated peaks of activity (Doy, Gresshoff and Rolfe, 1973). Since no intact bacteria could be isolated from the mycelium, the modified form of the mitochondria may indicate the possible location of the bacterial genetic information after transgenosis. This is supported by the observations of Swanson (1971) on the incorporation of high molecular weight polynucleotides by isolated mitochondria from *Xenopus laevis*. These polynucleotides served as templates for polypeptide synthesis on mitochondrial ribosomes. There also exists the possibility that the modified mitochondria found in the fungal strains capable of acetylene reduction represent L forms of the *Azotobacter* cells introduced into the fungal spheroplasts.

The decrease in acetylene reduction activity over an extended time period may have been due to the method of subculture. Only the younger portions of the mycelium were used to subculture the various strains which, if sectoring had occurred, may have resulted in the selection of portions of the hyphae with low activity. Sectoring may have been due to differential rates of mitochondrial and bacterial DNA synthesis and division during growth, but these rates of DNA synthesis were not determined.

There is an increasing body of evidence to suggest that bacterial DNA can be taken up by higher plant systems and the information it carries is available to the plant (Ledoux, Huart and Jacobs, 1971a,b; Doy, Gresshoff and Rolfe, 1973). If this method of transfer appears to be of more general and lasting application it may be possible to introduce nitrogen fixation into some of the vesicular-arbuscular endomycorrhizae, which can grow in the intercellular spaces of many higher plants, as well as the ectomycorrhizae. To be of direct benefit to the growth of higher plants, such a system requires that the subtle and complex relationship between both ecto- and endomycorrhiza and the host plant can be re-established after insertion of the bacterial information.

These results suggest that some degree of stabilization of the genetic information for nitrogen fixation derived from bacteria can be achieved within at least one eukaryote cell for a period of over 6 months provided sufficient selection pressure is present. Work is presently underway to determine the factors affecting the maintenance and stability of the genetic information within the eukaryote cell, and to assess the potential of similar nitrogen-fixing strains to reform a mycorrhizal association.

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61 Transfer of nitrogen-fixing ability to a eukaryote cell

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BIOTIC CRISES IN ECOLOGICAL AND EVOLUTIONARY TIME

edited by

MATTHEW H. NITECKI

Department of Geology
Field Museum of Natural History
Chicago, Illinois

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COMMUNITY EFFECTS OF INTRODUCED SPECIES

Daniel Simberloff

Department of Biological Science
Florida State University
Tallahassee, Florida

Though reported species introductions are a biased sample of all introductions, one may nevertheless fruitfully examine their consequences to cast light on community ecology theory. In particular, they would appear to be ideal tests for two broad classes of community models: (1) equilibrium island biogeography, and (2) limiting similarity. Examination of substantial lists of introductions shows little support for either the naive form of equilibrium island biogeography theory or its sophisticated variant, in which species replacement is predominantly within groups of ecologically similar species. Nor is there much evidence for limiting similarity. Both these observations march with similar results drawn from statistical examination of non-experimental systems, and point to the conclusion that interspecific competition, though viewed over at least two decades as the main force structuring communities in ecological time, is very difficult to demonstrate with data at hand.

Individual introductions certainly indicate competition, either between an invader and a resident or between two invaders. But even most of these studies are ambiguous, and for lists as a whole, the main observed effects of introduced species on communities appear to be generated by predation or habitat modification.

INTRODUCTION

Crises are usually so distinct from one another that one tends to view them idiographically, to seek unique explanations. The great prehistoric extinctions exemplify this tendency to explain apparently singular events idiographically, though a parsimonious explanation invoking nomothetic ecological principles is possible (Schopf, 1974; Simberloff, 1974a). Introductions of exotic species in various parts of the world are probably the most frequently cited crises in ecological time (*e.g.* Elton, 1958; Roots, 1976), and that so many introductions occur invites a search for nomothetic or even predictive laws, yet most treatments, even of lists of introductions, are idiographic. This is all the more striking since it would seem that observed consequences of introductions might bear on controversies currently surrounding community ecology (Simberloff, 1980), indeed might constitute the strongest evidence about some community theories.

I wish to examine the introduction literature systematically to see if one can conclude anything about two classes of community models: 1) equilibrium island biogeography, and 2) limiting similarity.

That limiting similarity and equilibrium biogeographic models seem such appropriate frameworks for examining the fate of introduced species is an interesting comment on the strength of the paradigm that nature is tightly organized into holistic communities. Both models are usually grounded in the assumption that the community is the fundamental natural unit, and the fate of any species, including an introduced one, rests on its interactions with and assimilation into a community (*e.g.* Diamond, 1975). One could as well have asked whether the community is important at all (or even exists as a holistic entity), and begun by focusing on individual species .

In fact, an entire book, *The Genetics of Colonizing Species* (Baker and Stebbins, 1965), took almost exclusively that viewpoint while looking at why some introductions succeed and others fail. But the vogue is now for community models (Simberloff, 1980) and to test two prominent ones will be my goal.

THE DYNAMIC EQUILIBRIUM MODEL

The dynamic equilibrium model of island biogeography posits an approximately constant number of species on an island, and claims the constancy is generated by a balance between immigration of species new to the island and extinction of species already on it. In its original form (MacArthur and Wilson, 1963, 1967) the model envisions the species in a pool as possibly having different propagule invasion rates or probabilities of extinction but does not otherwise distinguish or categorize them. That is, the original model predicts that immigration of a species will be compensated for by extinction of another species, but does not predict anything about *what* other species will go extinct. Even in the complete absence of interactions, an equilibrium number of species will obtain so long as each species has a finite propagule invasion rate and non-interactive extinction probability (Simberloff, 1969).

The original model quickly spawned many papers on the biotas of both oceanic and habitat islands (Simberloff, 1974b, 1976a), probably by virtue of its focus on a traditional statistic (species number) and its view of nature as dynamic but not too forbidding since it is divided into small units (Simberloff, 1978a). But from its inception the equilibrium model was usually not applied literally. Rather, two related provisos were often either implicit or explicit. First, interactions among species *do* occur (except possibly during the initial colonization of a sterile island) and may be important forces generating the extinction that drives the equilibrium. Simberloff and Wilson (1969), for example, proposed an "interactive" equilibrium number of species, lower than the initial "noninteractive" equilibrium on a newly colonized island; the decrease was a consequence of species interactions as populations increased. Wilson (1969) appended the notion of an "assortative" equilibrium, higher than the interactive equilibrium, that would arise as individual species' comings and goings by chance produce sets of species increasingly well-matched to one another with concomitant lower and lower extinction. Diamond's schema (1975) of "permissible species combinations" replacing "forbidden" ones by virtue of fuller use of available resources is a version of the assortative equilibrium. Finally is Wilson's "evolutionary" equilibrium (1969), still higher than the assortative equilibrium. A product of evolution over long periods, it results from the selective pressures species impose on one another. This evolution presumably leads to species that "fit" more compactly or efficiently on a given resource base, with niches that are minimally overlapping but maximally exhaust available resources: consequently more of them can coexist.

Closely related to and concurrent with the tacit or explicit incorporation of species' interactions in the equilibrium model was the recognition that interactions were more likely between certain pairs of species than others. Such recognition was implied at the outset by MacArthur and Wilson's examples (1967), which centered on given taxa rather than entire communities: birds and plants of Krakatau and plants of the Dry Tortugas. This tendency dominated subsequent applications of equilibrium theory to both real islands (*e.g.* Diamond, 1969; Terborgh and Faaborg, 1973) and habitat patches (*e.g.* Vuilleumier, 1970; Brown, 1971). Part of the reason, of course, was that species lists were usually available for individual taxa, not whole communities. But this was not disturbing since the taxa were viewed as more or less unified ecological groupings, and the tacit belief was that the one-for-one species replacements envisioned by the equilibrium model really ought to operate among ecologically similar species, not throughout the community at large (at least after an interactive equilibrium obtained). In short, interspecific competition, especially from related species, came to be viewed as a key force generating extinctions. One might well have expected this in light of the emphasis in recent evolutionary ecology on interspecific competition (*e.g.* MacArthur, 1972; Diamond, 1978) in spite of limited direct evidence that competition is, in fact, greatly significant in ecological time (Wiens, 1977; Strong *et al.* 1979; Simberloff, 1980).

The only formal incorporation into equilibrium theory of the notion that interactions are not community-wide but rather are mostly restricted to groups of ecologically similar species was that of Heatwole and Levins (1972, 1973). Their model stated that insular communities achieve an equilibrium community trophic structure independently of their approach to species-number equilibrium. That is, they claimed that the distribution of species into trophic categories such as herbivore or predator is an equilibrium characteristic determined by species' interactions and a community whose distribution is perturbed in some way will return to this trophic equilibrium. If true, this characteristic would constitute perhaps the strongest evidence that communities of animals are holistic entities and not just collections of species from among many possible, with rather similar physical environmental requirements: it would be a truly emergent property, not just a compound property (Salt, 1979). Unfortunately, Heatwole and Levins' means of adducing this equilibrium is statistically flawed (Simberloff, 1976b) and the observations can as well be explained by the null hypothesis that communities are random subsets of a large species pool than by the alternative that they are strongly structured by interaction. This is not to say that either hypothesis is in fact correct, only that the methods brought to bear so far (Simberloff, 1978b) do not necessitate rejection of the null hypothesis.

One may of course ask of introduced species, then, whether they produce changes in the invaded community that are explained well by the simple equilibrium theory and/or a more complex variant of it that envisions the one-for-one species replacement as occurring within ecologically circumscribed groups. For that matter one may ask simultaneously whether any other pattern of or generalization about community change induced by species introduction is manifested by literature descriptions.

LIMITING SIMILARITY

Theories of limiting similarity constitute an attempt to render the competitive exclusion principle more than just a trivially true epigram. That is, Gause's Law (1934) states that no two species occupy the same niche, but since two species are never completely identical, one can always find a niche dimension along which two species are separated: the law is not falsifiable.

Hutchinson (1959), in perhaps the most seminal paper in evolutionary ecology, suggested a related law that was at least falsifiable in principle: two species in the same trophic level cannot coexist if their sizes, or the sizes of their trophic apparati, are more similar than some critical ratio.

By induction on a few sets of vertebrates, Hutchinson suggeste that the critical ratio might be *ca.* 1.3; species more similar than this could not coexist because one would outcompete the other. Myriad papers have followed this lead, finding different critical limiting ratios permitting coexistence, and inferring that competition is too intense if sizes are more similar than this. Similarly, suites of more than two species are often claimed to have constant size ratios between pairs of species contiguous in a size ranking, with the constancy generated by competition. But since any set of numbers (or ratios) has a minimum, existence of a minimum ratio for some observed set of sizes cannot tell us why the minimum is as large as it is. In short, although Hutchinson's paper suggest a way to make limiting similarity a falsiable hypothesis, neither Hutchinson nor his followers have ever actually framed the question this way, or ever attempted to falsify an hypothesis.

Recently Simberloff and Boecklen (1980) attempted to correct this deficiency by asking whether there are any observations in the size ratio literature that are inconsistent with an hypothesis that sizes of coexisting species are random and independent of one another. In general, the answer was "No." In a few instances size ratios were too large (and/or too constant) to be construed as having resulted from chance alone; in a few, size ratios were too small (!) to be so construed, and for most systems sizes did not differ significantly from a random, independent model. This is not to say that sizes of coexisting species *are* random and independent, only that little in the existing published data says they are not. Once again, it would appear that observed success or failure of introduced species, plus their effects, if any, on the invaded community, might constitute a critical clue to questions of limiting similarity.

The simple model of limiting similarity relates to three others, two of them direct lineal descendents. Brown and Wilson (1956) described the phenomenon of character displacement, in which two species with narrowly overlapping ranges differ in size (or in size of some organ) more in the region of sympatry than do populations from regions of allopatry. The standard interpretation of this phenomenon is that it is engendered by selection to decrease competition so that it rests on the same belief as Hutchinson's: that there is some limiting similarity for sympatric species in the same trophic level. Grant (1972, 1975) has argued that the classic example of character displacement can as well be interpreted as individual species' clines, without recourse to species interactions. In any event, I have found no accounts of introduced species that suggest character displacement, and perhaps one ought not to expect the phenomenon to occur in ecological time. One may, of course, look for niche shifts even in the absence of morphological change.

Two attempts have been made to derive a limiting similarity model from first principles: a deterministic model (MacArthur and Levins, 1964, 1967 and many followers), and a stochastic one (May and MacArthur, 1972; May, 1973, 1974) that allows for stochastic variation in resources. Both begin quite explicitly by asking under what circumstances an introduced species can successfully insinuate itself into a community. But both are beset by assumptions that are unrealistic (Heck, 1976): homoscedastic, congruent resource utilization curves for all species, Lotka-Volterra population dynamics, negligible resource renewal time, a 1-dimensional resource continuum. And even if these were not unrealistic, it is scarcely conceivable that sufficient data could ever be gathered for any group of species to know that the assumptions were met. Certainly no such information is available for any introduced species and the community it has invaded. So the literature on introductions cannot be used to test these variants of the limiting similarity model. I am hard-pressed to think of *any* data that can test them! If introduction can be brought to bear on limiting similarity, it will have to be on the original, inductive model.

THE DATA

Two aspects of the literature vitiate conclusions that may be drawn about general effects of introduced species. First, available accounts surely constitute a biased sample of all introductions, in several ways. Species that survive in their new homes are far more likely to be reported than those that do not. Certain groups of species (birds, mammals) attract a disproportionate amount of study because they are of economic or aesthetic interest. Introduced species that produce spectacular effects, such as decreases in population sizes of residents, are more frequently reported than those that either quietly and quickly go extinct or unobtrusively gain a foothold. Further, there is no reason to think these sources of bias cancel one another with respect to testing either of our community hypotheses.

A second problem with the literature on introductions besets the ecological literature generally: inferences on species interactions are too frequently drawn without requisite consideration of alternative hypotheses to explain the observations. In many instances it was not feasible for more evidence to have been gathered, but one should then view conclusions as very tentative. Kitching and Ebling (1967) suggested that four criteria must be met before one can reasonably conclude that a predator is responsible for some species' absence:

1) One must know for certain, by prevention of predation, that physical factors are not limiting.
2) Geographic patterns of predator and putative prey must be complementary.
3) Laboratory or, preferably, field observations of predation.
4) Transplant experiments in which the predator is observed to eat the putative prey.

A similar set of criteria can easily be erected for interspecific competition. Criterion (2) (or its competition analog) is easily tested for introduced species, but Connor and Simberloff (1980) and Simberloff and Connor (1979) have shown how difficult it is to infer causality from biogeographic patterns alone. Criterion (1) is probably most often not tested. Criteria (3) and (4) are harder to examine for claimed competitors than for a putative predator-prey system.

A field experiment by Levins and Heatwole (1973) exemplifies how difficult it is to meet these four criteria and how easy it is to form conclusions without sufficient consideration of alternative hypotheses. They introduced frogs, lizards, snails, a *Drosophila*, and an ant onto a small sandy cay near Puerto Rico. The frogs, snails, and fruit fly quickly disappeared, and this was attributed to physical factors. The lizards and ant also disappeared but took a longer time to do it; this delay led to the conclusion that biotic forces (competition and predation) caused these exclusions. A population rather than a community model was elaborated to rationalize the former extinctions and to predict the fate of similar introductions. But the critical aspect of the model -- the extent to which the new physical habitat will lower the life expectancy of the invader below its optimum -- almost certainly cannot be determined without actually introducing the species, and maybe not even then. More seriously, whether to apply this population model or the community interaction model discussed above (Heatwole and Levins, 1972) is arbitrary; the tests suggested by Kitching an Ebling are not performed. Similarly, Carlton (1975) discusses the introduction of over 150 exotic marine invertebrates on th U.S. Pacific coast and observes a general dichotomy: most successful introductions are in estuaries, bays, or lagoons. Very few are on open rocky coast or sandy beaches. The main reason is said to be competitive: for historical reasons, the open coasts support a mature endemic fauna that tends to preclude invaders, while the estuarine areas, because they are smaller, geologically young, and widely separated, support a smaller, immature fauna that cannot prevent invasion. But virtually no experiments bear on this. This and the Levins-Heatwole explanations are scenarios rather than rigorously tested hypotheses, and represent what Ball (1975) has called the "narrative" stage of development of a science, an immatur stage in which competing explanations may all be more or less plausible but are not framed so they can be tested as null vs. alternative hypotheses. As Ball claims that progress in such a stage is limited, so I would add that we are not likely to understand the results of introductions unless falsification is attempted, probably by experiment.

Granted that these two limitations force us to interpret our findings tentatively, one can still see, in the introduction literature, at least a few good examples of any sort of situation. For an introduced predator that causes extinction of a resident, one may cite the introduction of the sea lamprey (*Petromyzon marinus*) into the upper Great Lakes after construction of the Welland Canal around Niagara Falls. Local extinction of the lake trout (*Salvelinus namaycush*) ensued (Smith, 1968; Christie, 1974, Magnuson, 1976).

Arrival of the alewife (*Alosa pseudoharengus*) on the scene brought about further changes in local species composition and abundance. For the sea lamprey's effect, all four of the above criteria are substantially met. Observe that if one were testing the simple equilibrium model, one would initially have seen a one-for-one replacement here, as theory predicts. But since the exchanged species are in different trophic levels, the sophisticated versions of the theory are falsified.

One can easily find examples in the introduction literature that implicate interspecific competition, though rarely with experimental evidence. One of the most impressive is that documented by Cameron (1958). The arctic hare (*Lepus arcticus*), originally Newfoundland's only hare, occupied both tundra, as on the mainland, and forest. Introduction of the varying hare, *Lepus americanus*, apparently caused the arctic hare to restrict itself to tundra. No extinction occurred, however. Further, an alternative, non-competitive explanation is possible (Birch, 1979). Extinction, apparently competitive, *is* known in ants. *Pheidole megacephala* replaced native ants on Bermuda and Madeira, but was subsequently extinguished itself by *Iridomyrmex humilis*, the "Argentine ant", on Madeira, and appears to be fading fast on Bermuda (Haskins and Haskins, 1965; Crowell, 1968). In spite of extensive field observation, no direct evidence exists on the nature of the competition so only criterion (2) is satisfied, but the dynamic nature of the boundary between the species' ranges certainly suggests interaction. Incidentally, *Pheidole*'s size exceeds that of *Iridomyrmex* by a ratio in excess of anything claimed as limiting in the limiting similarity literature. So if the competition were of the exploiting variety, one would have predicted coexistence.

One can also find reports of introductions that have no visible effects whatever. Abele (1972) records two: an Iraqui freshwater crab, *Neorhynchoplax kempi*, introduced into the Panama Canal, and a Brazilian freshwater shrimp, *Potimirim potimirim*, introduced into Florida. Doubtless many introductions that have no obvious effects are not discussed by ecologists, and unless they have taxonomic interest may not be reported at all.

But single cases, even if individually convincing, cannot lead to generalizations about how introduced species affect communities. The bias discussed earlier is one reason, but a more compelling one is the enormity of the literature and its generally anecdotal nature. In just 4½ months I have found over 1500 references to introductions. The vast majority are brief descriptions of events surrounding the introduction of one species or a few at one site. Mechanisms are rarely discussed, and range changes are emphasized. So I have instead focused on 10 review papers (Table 1), seven of which deal

Table 1. Reviews of introductions

Author	*Number of Introductions*	*Taxa*
Lever, 1979	*72*	*all vertebrate classes*
Greenway, 1967	*184*	*birds, mammals*
Nowak, 1975	*11*	*mammals, insects, crustaceans, molluscs*
Roots, 1976	*53*	*mammals, birds, amphibians, fishes, plants*
DeBach, 1974	*> 100*	*insects*
Holdgate & Wace, 1961	*14*	*mammals, birds*
Elton, 1958	*> 241*	*all vertebrate classes plants, insects, many other invertebrates*
DeVos et al. 1956	*> 135*	*mammals*
Courtenay & Robins, 1973	*44*	*fishes*
Courtenay et al, 1974		

exclusively with introduced species; each of the other three, though with different aims, treats a large number of introductions as part of another problem. For each paper I tabulated the number of species discussed and, from the review or a cited primary source, tried to determine what effects the invader had on its community. About 25% of the species occur on more than one of the ten lists. I must emphasize again that there is a clear tendency for all authors but one (DeBach) to focus on successful introductions, so evidence on how many introductions have failed (surely far more than succeeded) and why they failed is almost entirely absent.

RESULTS

The most striking result is that in so many instances (678 of 854), an introduced species has no effect whatever on species in the resident community, or on the structure and function of that community. Perhaps the second most striking result is the scarcity of extinctions apparently attendant on introductions (Table 2). A total of 854 introduced species produced only 71 documented extinctions of residents. Greenway (1967) cites most of these (55) of which over 90% are extinction of island species caused wholly or partly by introduced species. Most of these (82%), in turn, result wholly or partly from predation by an introduced species on a resident, while 20% are caused or facilitated by habitat destruction wrought by introduced species.

That fewer than 10% of these introductions result in a compensatory extinction can hardly be viewed as strongly supporting any form of the dynamic equilibrium model, though neither is it necessarily inconsistent with some versions of it. In particular, one can think of at least two circumstances under which one might have predicted that the equilibrium model holds, yet introductions need not incur quick extinctions.

Table 2. Results on species introductions

Author	Number of Introductions	Extinction of Residents	Extinction from Predation	Extinction from Habitat Change	Extinction from Competition	Possible Competition, no Extinction	Any effect at all
Lever, 1979	72	2	1	0	1	10	20
Greenway, 1967	184	55	42	7	0	3	60
Nowak, 1975	11	0	0	0	0	0	5
Roots, 1976	53	5	4	0	0	13	34
DeBach, 1974	>100	0	0	0	0	6	9
Holdgate & Wace, 1961	14	> 7	3	> 4	0	1	14
Elton, 1958	>241	0	0	0	0	3	4
DeVos et al. 1956	>135	2	0	0	0	13	25
Courtenay et al. 1973, 1974	44	0	0	0	2	2	5
	>854	>71	50	>11	3	51	176

First, the non-interactive model (Simberloff, 1969) predicts an equilibrium that is achieved probabilistically simply by many species' invading and going extinct independently, at species-specific rates. Chance events -- especially quick extinctions, long periods without invasion, etc. -- will dictate that the number of species may remain above or below the equilibrium for a substantial fraction of the time in any event, but an introduced species would simply temporarily increase species number by one without further effect on the resident community. Repeated introductions of the same species would, of course, change the equilibrium itself, since

$$\hat{S}_{\text{non-interactive}} = \sum_{\alpha=1}^{p} \frac{i_\alpha}{i_\alpha + e_\alpha}$$

where i_α and e_α are the species-specific invasion and extinction probabilities, respectively, and p is the species pool size. Repeated introductions of a species increase p by 1 and add a term to the equilibrium sum. A second conception of the dynamic equilibrium model that would not have predicted rapid compensatory extinction after introduction is if "relaxation" from a supersaturated state to a new equilibrium is generated by species interactions and is very slow. Diamond (1972) and Wilcox (1978) claim from existing species-area relationships for insular birds and lizards, respectively, that exactly this situation obtains, but Abele and Connor (1979) and Faeth and Connor (1979), respectively, have demonstrated that these claims are beset with statistical, geological, and biological flaws and unrealistic assumptions. The only direct tests of relaxation time, Levins and Heatwole's introduction study described above and my experiment on island insects, (Simberloff, 1976c) showed it to be very short.

Most of Greenway's islands are very small (*e.g.* Marianas Islands, Bonin, the Lesser Antilles), and in fact most of the

71 documented extinctions occur on small islands, where any delay in relaxation would be minimized. Furthermore, of the 71 extinctions, predation by introduced species is implicated in 50, and habitat destruction by introduced species is cited in at least 11 extinctions (the two categories are not necessarily exclusive; both forces could have operated). Competition from introduced species is cited in only three of the 71 extinctions (Table 2). In sum, then, these data certainly argue against any version of the dynamic equilibrium model tha envisions one-for-one competitive replacement within ecologica guilds or other circumscribed trophic units.

Of course, I must re-emphasize here that the reports from which the data are drawn constitute a very biased sample, and there is probably no way to tell how many introductions *failed* because of competition *from* resident species. Only three such instances are listed, all by Lever (1979) and all very tentatively: rainbow trout introduced into England may be outcompeted by native brown trout, as may the American brown trout. But no critical experiments have been done, and Lever cites other possible explanations for the failure of the introductic to spread. The possibility that native fishes outcompete the orfe is even more tentative. Interspecific competition *without* extinction is cited in only 51 of over 854 introduction episodes, and fewer than ten of these provide sufficient evidence that one feels confident that competition is, in fact, important. But interspecific competition, especially of the exploitation rather than interference variety, is surely a lower visibility phenomenon than predation, and failed introductions generate much less attention than partially successful ones.

In fact, the only conscious systematic attempt to document and to explain failed introductions is from the biological control literature, and it is distressing that generalizations do not spring to mind here. DeBach (1974) describes at least 100 introductions of parasites and predators in attempts to control 30 pests. For at least 11 of the 30 efforts, one or more of the introduced agents failed to achieve complete control because the physical environment -- particularly temperature and humidity -- was more inimical to the predator or parasite than to the host, at least over part of the host's geographic range. In only six of the 30 control efforts was interspecific competition implicated as limiting an introduction, and in five of these the competition was from a previously introduced species and not a resident. DeBach (1964) goes further, to argue that the shibboleth in the biological control literature (Pemberton and Willard, 1918; Smith, 1929; Utida, 1953), that one ought not to introduce two or more species to control one host because interspecific competition will render all ineffective, is falsified by observations. He finds not a single instance in which such competiton hampers control. This is not to say that competition does not occur among introduced species, even with subsequent extinction; five such occurrences are cited. But whenever this occurs the victor exerts better control anyway, and in any event myriad cases exist in which multiple introduced control agents coexist. Often each serves as primary control in that part of the host's range that is physically most suitable.

The biological control introductions when viewed as experiments are probably the most replicated and controlled, and certainly the most carefully monitored of all introductions, and serve as a warning about how difficult it is to determine without substantial pre-introduction data whether interactions are important. For example, extensive studies in England did not indicate parasitoids to be important controls of the winter moth, *Operophtera brumata* (DeBach, 1974). Yet introduction of a tachinid fly, *Cyzenis albicans*, and an ichneumonid wasp, *Agrypon flaveolatum*, from England achieved virtually complete control of the introduced Canadian population of winter moth. If one looked now in Canada, however, one would see an uncommon moth and two even less common parasitoids and would be hard-pressed to find a single parasitized moth. One would not likely deduce parasitism as the major population control of this pest without knowing the entire history of the introductions. The classic biological control example, control of prickly pear (*Opuntia* spp.). by the moth *Cactoblastis cactorum* in Australia (Dodd, 1940, Krebs, 1978), demonstrates the same point. Nowadays the moth is so uncommon, and its host not much more common, that it would take great insight to recognize that the former was responsible for purging most of a continent of the latter.

The evidence from introductions is hardly more supportive of the limiting similarity model than it is of dynamic equilibrium models. Price's attempt (1970, 1972) is the only one that explicitly treats an introduction in the context of limiting similarity, and the results are ambiguous. Four native parasitoids attacked a sawfly (*Neodiprion swainei*) in Canada; their ovipositor lengths produced three ratios as follow (species listed from smallest to largest):

Mastrus aciculatus
1.11
Pleolophus indistinctus
1.19
Endasys subclavatus
1.41
Gelis urbanus

Reasoning that differences in ovipositor length might well be a key means of partitioning resources among parasitoids, since they determine how deeply into the wood the parasitoids can attack their host, Price observed that it would appear more likely that an introduced species could successfully insinuate itself between the largest two species than elsewhere in the size ranking. In fact, a wasp (*Pleophus basizonus*) was introduced in the late 1930's whose ovipositor was intermediate in length between the two *smallest* wasps, and produced the sequence of size ratios 1.05, 1.05, 1.19, 1.41. No species was extinguished, and *P. basizonus* survived well, but Price still views the result as supporting a model of limiting similarity. This is because, at high densities only, the two species closest in size to the introduced one parasitize a smaller proportion of the total parasitized sawflies than do the two larger species, or than does *P. basizonus* itself. Why this should indicate that the species compete, or that the introduced species competes most strongly with the two most similar to it, is unclear. There is no evidence that the curve of proportion parasitized *vs.* host density would be different for the two putative competitors were *P. basizonus* absent. All we know is that the density-dependence of *M. aciculatus* and *P. indistinctus* is limited to low host densities; but so are those of many species. And that this particular pair have the poorest response of all four residents at high density is not astonishing; there are six pairs, so by chance alone one would expect this pair to be least responsive 17% of the time, even if similarity to the introduced species had no effect.

Price (1975) suggests that a consideration of Hutchinson's limiting similarity model could have allowed one to predict the outcome of the introduction of *P. basizonus*. The results of the biological control introduction cited by DeBach (1974) do not support this contention. In slightly fewer than half of the 30 situations, two or more introduced parasitoids that differ in linear size by less than a factor of 1.2 survived and continue to coexist. But no general conclusion can be drawn, since one can also find several cases in which one member of a pair of similarly-sized species was not successfully established. Nor do the other cited introductions support the limiting similarity model, since many examples are seen in which a species colonizes successfully when a similar sized confamilial or even congeneric species is present.

CONCLUSIONS

What can we learn from this survey? First, and a bit depressingly, to be humble about the ability of current ecological theory to predict the outcome of introductions, and probably of crises generally. We see that two very general and venerated community models do not permit us to intuit what will happen after an introduction, and the results do not cause useful generalizations to leap to the mind of one ecologist, at least. I have sought such generalizations from a community standpoint, and mathematical models abound that purport to generate such generalizations and predictions. As I have discussed earlier, some of these have such unrealistic assumptions that one would not expect them to provide insights, others require parameters that we cannot hope to measure in nature. The two community models that we specifically examined -- dynamic equilibrium biogeography and limiting similarity -- clearly do not predict or even explain the results of introductions, although in a statistical sense for large enough biotas the former may still be an accurate general description. Perhaps the community is not the appropriate level for us to focus on; maybe it is but an epiphenomenon of component population processes (Simberloff, 1980).

But it is interesting to note that a review of introduction literature in biological control from a population standpoint led DeBach (1965) to a similarly modest conclusion: "An analysis of some 225 world-wide cases of partial or complete biological control by imported entomophagous insects fails to reveal any obvious common denominator having a genetic, biological, or phylogenetic basis which would be predictive of post-colonization results in a new environment even assuming sound biological and ecological judgment had been used." Despite the lack of predictive models, and the fact that one can find some examples of almost any possible outcome of an introduction, DeBach (1971, 1974) is proud of biological control's accomplishments and sanguine about its future. About 25% of such efforts have succeeded in the past in spite of rudimentary technology, and there is every reason to think this figure can be raised. General guidelines can be given (DeBach, 1965), such as introducing insects only if the native climate is similar to the intended new home, but one ought not to aspire to a high level of accurate predictions.

Elaborate and precise community models for the trajectories of introductions may similarly constitute attempts to see how many angels can dance on the head of a pin. General guidelines may be given -- predation is likely to be important, probable habitat modification may be obvious, and again climate must be suitable -- and detailed preliminary field work can help to predict and therefore to avoid disasters. But the organisms themselves will likely frequently conspire to confound our hardest earned expectations. Strong (1980) has suggested that the uniqueness and great number of ecology's fundamental units (populations and individuals) compared to those of physics and chemistry doom our attempts to emulate the precise and elegant models of the physical sciences. "Physics-envy is the curse of biology" (Cohen, 1971) and we ought not to emulate the methods and criteria for success of the physical sciences (Slobodkin, 1975; Simberloff, 1980). As the experience of biological control teaches, we may nonetheless successfully solve practical problems in ecology, including some of those generated by the ability to introduce species.

To show how idiosyncratic introductions may be, and to attempt to transmit to you an inkling of the wonder and surprise that must constantly fill an ecologist studying them, I close with a few examples. The Lanai thrush (*Phaeornis obscurus lanaiensis*) was probably extinguished when introduced mosquitoes transmitted to it introduced diseases from introduced poultry (Greenway, 1967); diseases and a perverse vector may often fatally link species that one would never have expected to interact.

Native Hawaiian moths have declined in numbers and some have even been extinguished; it is likely that much of this is due to ichneumonid parasitoids themselves introduced to control primarily introduced pests on crops that are themselves mostly introduced (Elton, 1958). Three of these parasitoids (*Casinaria infesta, Cremastus flavoorbitalis*, and *Hyposoter exiguae*) are particularly polyphagous, and their reduction of moths may be responsible in turn for reduction of native bunting wasps of the genus *Odynerus*, which primarily feed their larvae on moth caterpillars.

An unsuccessful effort was mounted in Florida to prevent introduction of the grass carp (*Ctenopharyngodon idella*) to control various introduced weeds that clog our waterways (Courtenay and Robins, 1973) because of its effect elsewhere on waterfowl and sport fishes. Its destruction of habitat may lead to reduction or elimination of several species. The fish is currently spreading in Florida but no extinctions are yet documented. Habitat alteration often mediates species interactions, and since many species use different habitats for different functions or at different times in their life cycles, potential interactions of this sort are myriad. Of course species' reductions may then have cascading effects through entire communities.

Finally, a mite (*Pediculoides ventricosus*) was accidentally introduced into Fiji (DeBach, 1974), where it attacked larvae and pupae of the coconut leaf-mining beetle (*Promecotheca reichei*) but not eggs or adults. In the dry season it locally destroyed all larvae and pupae; the beetle adults then laid eggs and died, so a beetle population with overlapping generations was converted to a synchronous population. But now there were no larvae and pupae, so the mite population plummeted. Meanwhile the synchronous nature of the beetle population had virtually eliminated two native parasitoids that had previously controlled the beetle; their generation lengths were too short for them to survive the intervals between occurrences of the restricted host stages in which they oviposit. Previously there had been beetles of all stages present at all times. So now there was a beetle explosion. This story has a happy ending, since a parasitoid not so fussy about the stage in which it oviposits was successfully introduced from Java (where it is neither common nor particularly important to a native *Promecotheca*) and controlled the scourge (Taylor, 1937). Perhaps this will end my review on a happy note: no model predicted the original problem, nor indicated exactly what to do about it. But careful autecological study of the four key species led to a search for a parasitoid with quite specific traits, and turned tragedy to triumph.

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APPENDIX B

DRAFT

Regulation of Genetically Engineered Substances under TSCA

3
March 198~~2~~

DISCLAIMER

This document is a preliminary draft, and has not been peer and administratively reviewed within EPA. It should not be construed to represent Agency policy.

Chemical Control Division
Office of Toxic Substances
Office of Pesticides and Toxic Substances
United States Environmental Protection Agency
Washington, D.C.

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1. Introduction

The purpose of this paper is to present the major issues which must be investigated in order to determine whether the Office of Toxic Substances (OTS) has or should establish regulatory responsibility in the developing area of biotechnology.

The paper is not intended to answer the numerous and very complex questions of how TSCA might be implemented in this new area. Rather, it establishes that from a preliminary standpoint there appear to be good reasons for OTS to assume some level of regulatory responsibility for biotechnology, and that extensive investigation will be necessary to work out the details.

In summary, this paper provides the following:

- a brief history of the biotechnology industry,
- a broad discussion of the nature of health and environmental concerns about the industry,
- an overview of the issues which must be analyzed in order to determine TSCA's role in the biotechnology area,
- resource and information obstacles if regulation under

TSCA is eventually to be established.

* * * * *

There is no generally accepted definition of biotechnology. The Organization for Economic Cooperation and Development (OECD), after reviewing ten different definitions, proposed using "the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services."

For purposes of this paper, biotechnology will be defined as the manufacture, processing, distribution in commerce, use or disposal of genetically engineered substances. 1/ Thus, regulation of biotechnology could encompass any commercial or industrial activity having to do with genetically engineered substances themselves, as well as products produced by genetically engineered substances.

Biotechnology is an evolving field in which other government agencies, private and non-profit groups (including universities, major chemical and pharmaceutical companies, and genetic engineering firms) have become increasingly involved. The OTS

1/ Genetic engineering is the process of purposefully changing the hereditary material (DNA) of a living cell(s), as opposed to natural selection or mutagenesis which may occur without purposeful human intervention.

role has not yet been defined. There are questions of whether some substances in this field may be subject to TSCA jurisdiction at all, and there is no clear view either of the potential risks of such substances or the appropriate approach to their evaluation.

Since the development of the NIH guidelines on research practices in this field, EPA has expressed concerns about the potential environmental impacts and health risks associated with commercial activities, neither of which were covered by NIH. However, no EPA position has yet been formally stated or implemented.

At the March 1982 OTS Program Review, the Director of OTS requested that a paper on biotechnology be developed, to serve as the basis for the Office to decide what additional actions, if any, should be taken. In September, 1982, the Assistant Administrator for Pesticides and Toxic Substances asked the Administrator's Toxic Substances Advisory Committee to take a long-term look at the kinds of issues the Agency might have to address if it became involved in regulating biotechnology.

2. Background Relevant to TSCA

Basic research on genetic engineering advanced rapidly in the 1970s, and its quality has been recognized by the award of several Nobel Prizes to American scientists.

U.S. industry has begun to contribute to biotechnology research and to capitalize on its results. Large drug, chemical, and agricultural companies have entered into agreements with universities, invested in genetic engineering firms, and created their own biotechnology divisions. Dow, DuPont and Monsanto are among the chemical companies that are increasingly invested in biotechnology. While basic research is thus continuing, there is increasing likelihood of commercial applications.

It is possible to identify a number of current and potential commercial applications that could involve the use of genetically altered microorganisms or other living systems. These include the following examples (Part 5 will separate out the specific uses or commercial activities which could fall under the jurisdiction of TSCA):

Drugs--Hormones may be produced in sufficient quantities, by using biotechnology methods, that other production methods (such as chemical synthesis and extraction from the glands of dead humans and animals) would be replaced.

Food processing--Single cell protein, from such products as waste sawdust and methanol, may be produced more efficiently.

Mining--Microbial leaching of minerals from slag may be improved.

Oil--Xanthan gum and microorganisms for enhanced oil recovery may be used.

Pollution control--Microorganisms may be used to degrade oil for cleanup of oil spills.

Agriculture--Genetic engineering could be used to enhance nitrogen fixation capabilities of crops, possibly leading to increased production with less use of fertilizer, in animal husbandry, etc.

The greatest relevance of TSCA to biotechnology is to potential applications in the chemical industry. The following characteristics of biotechnology lead to the promise of wide application in this industry: the ability to use renewable resources as feedstocks, the ability to use lower temperatures and pressures (and therefore less energy) in production processes, the ability to simplify serial chemical processes into one-step biological processes, and the expectation of less pollution because of end-product purity and fewer undesirable by-products.

Although fermentation processes have been used for centuries in the production of foods and drugs, the chemical industry is largely petroleum based and used to dealing with the physical sciences and engineering. For this reason, the chemical industry is behind the drug industry in the application of biotechnology. Therefore, although applications that could be subject to regulation under TSCA may already exist or are near-term possibilities (e.g., genetically-engineered oil spill degraders), widespread application of biotechnology in the chemical industry (i.e., major changes in the methods of production) may not occur for some time. Of course, applications which would require TSCA jurisdiction cannot be accurately predicted at this point in time. Therefore, applications must be continually monitored as they evolve, in order for OTS to anticipate its future responsibilities.

4. Potential Health and Environmental Concerns

The major public fears associated with genetic engineering are that purposefully or accidentally, DNA will be changed through means and/or with outcomes that are unacceptable to society. An additional fear is that new strains of microorganisms will be developed that may have adverse or even catastrophic effects on humans or the environment.

The principal early concerns among researchers on DNA were the potential health risks (both toxic and pathological) posed in laboratories as the consequence of direct exposures to genetically engineered organisms. The major actions taken to protect against adverse health or environmental effects in laboratory research on recombinant DNA have been physical containment of the substances and the use of attenuated strains of bacteria. Approved disposal and worker protection methods have also been developed as a result of legislative review, NIH guidelines, and creation of institutional biosafety committees in organizations receiving federal funds for recombinant DNA (rDNA) research. Thus, guidelines and oversight functions have been the main lines of defense for the potential risks of this technology to date, and these have focused exclusively on research-related risks.

The use of genetic engineering is moving increasingly from research to industrial application. This means that the products and uses of microorganisms are moving from small-scale settings (containers on the laboratory bench) to large-scale development and manufacturing, and eventually to applications in plants and in the environment. Thus, current controls may not be adequate to meet the potential risks to health and the environment from purposeful or inadvertant exposure to rDNA substances. The following are some general concerns:

(i) Use of more virulent strains. The trend in biotechnology research is towards less concern about the virulence of the strains used. This is partly because experience has not led to serious negative consequences, and partly because the health hazards and appropriate safeguards appear to be less than those used in research on rabies, typhus and other virulent substances. Most rDNA research has been done on strains of *E. coli.*, a bacterium that is found in animal (including human) intestines. Researchers are beginning to investigate using free-living and spore-forming bacteria, and genera that are human, plant or animal pathogens. There is limited information concerning the survival of engineered organisms or the likelihood of their genetic exchange with other life forms, but there is the possibility that they could infect or cause toxicity in non-target species, including humans.

(ii) Research not subject to NIH guidelines. Only federally funded (mainly NIH and NSF) research must be in compliance with the NIH guidelines. However, a number of firms and laboratories which are not federally funded have voluntarily agreed to abide by the guidelines, and some regulations have been passed by State and local governments. (Cambridge, Massachusetts, passed a law that genetic engineering firms in that city must adhere to the NIH guidelines.) Since the availability of private funds for research is expected to increase, along with a tendency toward increased secrecy on the part of companies competing to develop new products, we may face in the future a greater degree of independent, unmonitored research and development activities.

(iii) Inexperience with large-scale production. As companies that do not have experience with strict containment and other protections start producing biotechnological substances or products on a large scale, there are increased risks of accidents or mistakes that could lead to inadvertent release of genomes, and exposure of workers, consumers, the public and the environment. The risks and needed protections may be far different from the traditional fermentation industry.

(iv) Intended dispersal in the environment. The protective procedures and mechanisms that have been developed so far have concentrated on containment, and specific health effects on humans. A number of the potential applications of DNA involve deliberate dispersal into the environment. Whether attenuated strains are used or not, there will often be new strains for which humans may have no existing defense against adverse effects. Additional unknowns arise when considering potential impacts on existing ecosystems.

b. Examples of biotechnology risks

Examples of specific risk scenarios that could develop in various industries include the following (some of these problems are also associated with conventional microorganisms):

Drugs--There is the risk of exposure of workers and the surrounding community to aerosols containing microorganisms developed from human pathogens, which might be able to cause infection. New genetic engineering firms do not have the experience of established drug companies in the use of containment procedures. (Furthermore, FDA does not have regulatory jurisdiction over drug manufacturing. OTS should therefore consider whether it has, or should establish, jurisdiction in this area.)

Chemicals--Potential risks are qualitatively similar to those seen in the drug industry. However, lack of experience in the use of containment procedures, and the greater potential scale of such operations, may increase the potential for worker exposure and environmental release of genetically engineered organisms.

Energy--Such applications often involve the direct release of genetically altered organisms to the environment. As in the chemical industry, the microbes used in enhanced oil recovery are potentially serious human pathogens.

Mineral leaching from slag heaps--The large-scale use of microbes may result in natural selection of bacterial strains that are infectious to humans. The leaching may also enhance the generation of sulfuric acid which could cause serious acidification of fresh water sources.

Metal concentration from settling ponds or dilute water streams--The use of bacteria could transform some of the metals (e.g., mercury) into organometallic compounds that are toxic to higher life forms and could enter the food chain in the environment.

Waste treatment--Heavy metal ions might be transformed by microbes into organic derivatives that are toxic to aquatic animals that take them up from the sediments. In other applications there is a potential public health threat from infectious bacteria being spread through aerosols generated by sewage treatment plants (i.e., from air bubbled through activated sludge and sewage water splashing over rocks in trickling filter beds). Similar risks exist for conventional biological treatment facilities, but the use of genetically modified organisms may increase the concern for such risks.

Agriculture--Sewage and other forms of wastewater might be treated by genetically altered organisms and applied as fertilizer to croplands. This could result in harmful aerosols and groundwater contamination. There are also risks that genetically engineered species could transfer genetic material to other plants, perhaps resulting in more vigorous weeds (resulting in a need for more herbicides), increased denitrification, increased crop disease susceptibility, and changes in the niches and pathogenicities of plant viruses and soil bacteria.

In conclusion, this section has discussed some general and some specific concerns about the risks associated with the use of biotechnology. It should be emphasized that there is no way yet to fully characterize these risks (if any) nor is it clear that these risks are significant either in absolute terms or in relation to the potential benefits of biotechnology. The uncertainty about the likely nature and magnitude of these risks is very great and is one of the major factors for OTS to consider in exploring its policy options.

4. Jurisdiction of TSCA

There are a number of complex issues that relate to the jurisdiction of TSCA over biotechnology. Some of these issues are discussed below, but definitive conclusions about TSCA's statutory authories will require extensive research and are not possible based on this degree of analysis. Nonetheless, there appear to be many reasons for OTS to assume some level of regulatory responsibility for biotechnology.

a. Are genetically engineered substances "chemical substances" under TSCA?

The first issue that must be addressed is whether genetically engineered substances are "chemical substances" under TSCA and are therefore subject to regulation under the law.

Section 3(2)(A) of TSCA states that, except for the exclusions in §3(2)(B), "the term 'chemical substance' means any organic or inorganic substance of a particular molecular identity, including—(i) any combination of such substances occurring in whole or in part as a result of a chemical reaction or occurring in nature, and (ii) any element or uncombined radical." The exclusions in §3(2)(B) include: (i) any mixture; (ii) any pesticide; (iii) tobacco or any tobacco product; (iv) any material subject to the Atomic Energy Act of 1954; (v) any article the sale of which is subject to the tax imposed by §4181 of the Internal Revenue Code of 1954; and (vi) any food (including poultry and poultry products, meat and meat products, and eggs and egg products), food additive, drug, cosmetic, or device.

It seems useful to define biological substances on an _a priori_ basis as including the following three types.

(i) _Simple living organisms (microorganisms)_. By being simple (e.g., including yeasts, fungi, bacteria, and viruses, but perhaps not some forms of algae or protozoa), this type excludes any organism complex enough to be identifiable as a "higher" animal or plant. There are two sub-types of microorganisms--those that are naturally occurring and those that are modified by genetic engineering. This differentiation has implications both for the Inventory and for submission of PMNs and is discussed further below.

(ii) _Derivatives of plants, animals, and micro-organisms_. These are organic substances produced by and/or derived from living or once living plants, animals and microorganisms. There are implications for Inventory reporting and PMN submission based on the method of derivation.

(iii) _Synthetically produced organic substances that are the equivalent of functional components or extracellular products of plants, animals, and microorganisms_. Examples would be synthetic polysaccarides or enzymes produced by genetically-engineered microorganisms.

Biological substances can be naturally occurring (feedstocks, intermediates, catalysts) or they may be products and byproducts of genetic engineering.

Biological substances that are type (ii) or type (iii) (i.e., living organism derivatives, or synthetically produced organic substance equivalents), are clearly chemical substances under TSCA if they are manufactured for TSCA purposes--i.e., purposes that are not excluded by the law (e.g., pesticides, food, drugs, etc.).

The key issue is whether type (i) biological substances (e.g., microorganisms), when used for TSCA purposes, are also chemical substances under the law. There are strong arguments for considering them to be so. First, TSCA is very clear in being all-inclusive in its definition of chemical substances; the exclusions, aside from mixtures, are specific references to substances regulated under other Federal laws. Second, the legislative intent of TSCA was to be the "gap-filler," so that any substance not adequately regulated under other Federal laws would fall under TSCA jurisdiction. Third, §3(2)(A) states that "the term 'chemical substance' means any... combination of (organic) substances... occuring in nature...." Microorganisms may be substances, even if modified by genetic engineering.

Finally, there is the precedent of including Unknown or Variable compositions, Complex reaction products, or Biological materials (UVCB's) on the Inventory. Several classes of such materials, including bacteria, fungi, yeasts and microorganisms, are listed on the Inventory. The Inventory reporting instructions included guidance on the reporting of naturally occurring substances such as bacteria, yeast and fungi.

Although it appears that living organisms are chemical substances under TSCA, it is somewhat novel to interpret TSCA for such substances, because it has always been interpreted in the context of non-living matter. Terms such as manufacture, process, chemical identity, molecular structure and exposure take on unique meanings when applied to living organisms. Other considerations associated with review and regulation of these substances, which will be discussed in the following parts of this paper, will also contribute to a decision on the role of TSCA for these substances and/or organisms.

b. Inventory policy

The manner in which a chemical substance is treated on the Inventory defines to a large extent whether the substance is considered "new," thus requiring a PMN under TSCA. Naturally occurring chemical substances (e.g., plants and animals) are considered to be on the Inventory even if they are not specifically listed. Thus, individuals who obtain such materials from nature (e.g., lumber, gravel, mineral ores) are not required to submit PMN's. Because of this provision, naturally occurring microorganisms would automatically be considered to be implicitly on the Inventory.

OTS has examined the status of organisms which a) exist in nature but are cultured and grown for commercial purposes, and/or are derivatives of naturally occurring substances, and b) are not specifically listed on the Inventory. According to TSCA, if such organisms are unprocessed, or processed only by manual, mechanical, or gravitational means, they are implicitly considered to be on the Inventory. For example, an enzyme extracted from naturally occurring substances by manual, mechanical or gravitational means would, itself, be considered naturally occurring.

If the derivatives of naturally occurring substances are produced by means such as organic solvent extraction (which might have the potential for affecting their chemical composition and therefore their toxicity), they are not automatically listed on the Inventory and, if manufactured for TSCA uses, are subject to PMN requirements. Thus, an enzyme which is extracted from vegetable matter by means of a hexane solvent would be subject to PMN if the enzyme were not specifically listed on the Inventory.

Many organic substance equivalents can be produced synthetically. If these substances are not specifically listed on the Inventory, they require a PMN if manufactured for a TSCA use. This would include type (iii) biological substances. There is a subsidiary issue, however, which is whether the same polypeptide produced by a naturally occurring or a genetically-engineered microorganism would be considered equivalent on the

Inventory to the extent it is a complex or biological substance. In the case of UVCBs, where the source is important in characterizing the substance, the genetically engineered polypeptide probably would not be considered naturally occurring for Inventory purposes. However, this is another complex issue which cannot be fully resolved without further analysis.

Currently there is no Inventory policy regarding genetically engineered microorganisms. The Inventory Team has received some inquiries about whether such substances should be reported, but has deferred giving answers until a policy decision is made by the Office.

c. Role of TSCA in research and development

TSCA provides EPA with limited authority to regulate research and development in manufacturing and processing of chemical substances so long as it can determine a reasonable basis for concern. EPA also has responsibility for coordinating and conducting research in the area of chemical risk assessments, including the development of appropriate test methodologies. The implications of these TSCA authorities for biotechnology are discussed below.

Congress provided EPA with authority to regulate research and development. It limited this authority, however, since it did not want to overly impede innovation and recognized that there are fewer risks generally associated with research and development than with later stages of manufacture and use. Section 8(b) excludes from the Inventory chemical substances manufactured or processed in small quantities for research, analysis, and development of a product. Section 5(h)(3) also excludes them from the requirements of a PMN. In both cases the law leaves the determination of "small quantity" to the Administrator. Generally this has not been specifically defined, but taken to mean any amount of the substance as long as this amount is no greater than necessary to do the research. However, where microorganisms are tested in an ocean environment to determine their efficacy in degrading oil slicks, or where they are injected into oil wells to see how well they enhance oil recovery, this could involve a significant quantity of material. Furthermore, even small numbers of such organisms could multiply, in a favorable environment, to produce a significant population of potentially hazardous organisms. The risks of such activities may, for this reason, also be significant.

Section 5(h)(3) does give the Administrator the authority to prescribe the manner in which those who do the research and development must be notified of "any risk to health which the manufacturer, processor, or the Administrator has reason to believe may be associated with such chemical substance." While the final PMN rules prescribe the manner in which such notification must be made, the adequacy of such notification, given the potential risks of rDNA substances, has not been evaluated.

The situation could well arise in which significant quantities of living organisms resulting from research are distributed in commerce in "small quantities" for R & D purposes. It is an issue whether such activities and the resulting laboratory (company or university) research could or should be subject to regulation under §6 of TSCA. While some risks may be adequately covered by the NIH guidelines, these guidelines do not consider potential environmental effects. Uncertain risks associated with some of the products of biotechnology research (for example organisms which can reproduce in the environment) might warrant their exclusion from the proposed low-volume exemption. Because of the uncertainties associated with their toxicity, biopolymers were specifically excluded from the proposed polymer exemption.

Unlike the previously cited sections, §6 of TSCA provides no exclusion for research and development where the Administrator finds that such activities will present an unreasonable risk. Thus, the Agency has the option to take action under the authority of §6, so long as it can determine a reasonable basis for doing so.

In the area of chemical risk assessments, the Administrator can work with the Secretary of HEW under §27 to conduct or finance projects "for determining and evaluating the health and environmental effects of chemical substances and mixtures, and their toxicity, persistence, and other characteristics which affect health and the environment...." EPA's Office of Research and Development (ORD) is currently carrying out some research that could fall under this category, but the Agency could do significantly more research on the risks associated with TSCA uses of biological substances, and methods to determine the hazards and to assess the risks involved.

d. Role of TSCA in manufacture, use, workplace hazards, and environmental contamination

To the extent biological substances are chemical substances and used for TSCA purposes, the law provides authority to review health and environmental impacts throughout the entire life cycle associated with their use (except for specifically excluded cases; refer to p.24). For example, determining acute and chronic health effects of chemical substances on workers during manufacture and use is a traditional area of TSCA concern in reviewing new chemical substances. The type of review would be the same for biological substances, except that the analyses and types of concerns would be different where living organisms themselves are involved. Environmental contamination and its consequences are the largest unknowns in the use of genetically engineered microorganisms. TSCA should probably play a key role in the evaluation and regulation of such risks.

5. Role of Other Agencies and Authorities vis-a-vis TSCA

There are three considerations in determining TSCA jurisdiction. One is statutory authority, another is need for OTS involvement, and a third is the jurisdiction of other EPA and governmental authorities.

a. Authorities that exclude TSCA

Section 3(2)(B) of TSCA provides guidance as to which substances are excluded from regulation under TSCA. Among biological substances the most important exclusions are those which are regulated by the U.S. Food and Drug Administration (FDA) and EPA's Office of Pesticide Programs (OPP). However, FDA and OPP have authority to regulate products but not their manufacturing. Thus, a microbe that is genetically engineered to produce a drug or pesticide may be subject to regulation under TSCA. Also, substances regulated by these other offices but then used for TSCA commercial purposes would come under TSCA jurisdiction for those uses.

b. Other agency authorities permitting a TSCA role depending on need

Section 9 of TSCA provides that the Administrator may provide a report to other federal agencies based on a finding of risk. The report must request the other agency to determine whether the risk could or should be reduced by taking action under own laws. If the other Agency agrees that there is a risk and initiates action, or if the agency declares that the risk does not exist, EPA may not take action independently. Section 9 also requires the Administrator to consult and coordinate with the heads of other federal agencies to avoid duplication of effort; other federal laws are to be used to protect against possible risk unless the Administrator determines that actions taken under TSCA would be in the public interest.

Some of the agencies that have played or could play a role in the area of biotechnology include the following:

(i) NIH. The NIH guidelines, as revised, cover laboratory research that is federally funded. As noted earlier, many firms and some local governments are also voluntarily complying with them. The Recombinant DNA Advisory Committee (RAC) 2/ has been extending its work to cover large-scale research and some industrial practices, alhough RAC has acknowledged that it does not have expertise in assessing environmental risks. There is a continuing controversy over the extent to which NIH and other authorities should or will become involved in developing guidelines not only for research, but for commercial development and scale-up of biotechnology.

2/ A committee formed under the auspices of NIH to investigate and report on health risks associated with genetic engineering research.

(ii) OSHA. OSHA has regulatory responsibility for worker safety--both in promulgating laws and in doing inspections. It has not been active, however, in the biotechnology field.

Section 9 of TSCA has been interpreted to mean that EPA action will not preclude OSHA action. However, EPA is not precluded from issuing regulations which affect occupational safety and health. Also, EPA has authority under §5 to take action because of concern for workplace risks, whereas OSHA's authority requires demonstrable risk. Therefore, §9 does not preclude OTS regulation of occupational risks of new chemicals. Futhermore, biotechnology should fall under §5 provisions because it will involve "new" substances. Therefore, there is good statutory justification for OTS to analyse the risks involved and, coordinating with OSHA, see that adequate protections are prescribed.

(iii) NIOSH. NIOSH provides back-up research and makes recommendations to OSHA, but does not itself have regulatory authority. Under the previous Administration, NIOSH attempted to develop an rDNA program. It did a series of "walk-throughs" to begin learning about industry practices and to serve as the basis for recommendations to OSHA. It also had a contract with EPA (which was aborted) to look at fermentation and extraction procedures. Under the current NIOSH priority-setting system, however, biotechnology is not included as an activity. NIOSH only has a staff member attending RAC meetings as an observer, and through CDC it sponsored the ad hoc working group on medical surveillance mentioned above.

Additional work should be done to define the appropriate federal role in analyzing the potential risks, or determining possible protections against risks to health or the environment, from non-laboratory uses of rDNA products. It is far easier in a new than an established industry to educate managers, engineers and other workers about the risks and protections needed--before major investments are made rather than afterwards. Federal agencies could play a major role in bringing this about.

c. TSCA jurisdiction vis-a-vis other EPA authorities

Section 9 of TSCA states that the Administrator shall coordinate action taken under TSCA with other laws administered by the Agency. Within EPA there are several other authorities that could have responsibility for aspects of biotechnology--e.g., air, water and drinking water. The Office of Solid Waste has developed a Draft Manual for Infectious Waste Management (Sept., 1982) that could be used to develop guidance under TSCA for handling, treating and disposing of living organisms which may have pathogenic characteristics. OPP has proposed data requirements for biorational pesticides that could be very useful to OTS, as discussed elsewhere in this paper. ORD is funding several projects related to biotechnology or protecting and enhancing the environment; it has also provided a representative to RAC, is sponsoring a series of evening seminars on biotechnology with the AAAS, and will co-sponsor a conference on the application of genetic engineering to pollution control.

In general, however, there have not been attempts by other EPA offices to take a lead for the agency in the area of biotechnology. The informal working group set up by the Science Advisor to the Assistant Administrator for Pesticides and Toxic Substances is increasing the level of communication in the agency on this topic.

The Office of Toxic Substances has begun a concerted effort to: 1) analyze biotechnology issues, 2) establish and coordinate Agency policies with respect to genetically altered substances, and 3) implement these policies in OTS. OTS appears to be the appropriate office to lead the Agency in examining EPA's eventual role in this important area.

6. Implications for Review and Regulation under TSCA

a. PMN process

Genetically engineered microorganisms would be a completely new category of chemical substance for review in the PMN process. Many of the standard approaches to the review of "conventional" chemical substance would not be applicable. The problem is that there would not be a base of data or experience from which to draw conclusions. Even if the structures of genetically engineered organisms were exactly specified (highly unlikely), there would be no analagous substances from which inferences could be drawn. If the structures could not be precisely specified, as would frequently be the case, the uncertainty of any inferences would be enormous.

Structure activity analyses, which form the backbone of the PMN review, will not be sufficient for analyzing risks of living organisms. Therefore, OTS would have to treat each substance on a case-by-case basis. Because the number of such substances submitted is not likely to be large in the near term, the building of an empirical OTS database would not proceed quickly. The best OTS can do is to stay abreast of industry developments to maximize the lead time to prepare for PMN submissions. For example, if it seemed that a PMN would be submitted for a substance or micro-organism that degrades oil, staff could examine the literature and talk with researchers in the field to prepare themselves. A key issue for OTS to resolve is whether, how, and what types of testing to require under §5(e) if significant concerns are identified.

b. Test data development

OTS must anticipate that PMN submitters will seek guidance on appropriate testing, because one of the serious deficiencies in assessing the risk of genetically engineered substances would be the lack of relevant test data on either health or environmental effects. The current OTS test guidance was developed for traditional chemicals; it is not appropriate to developing test data on genetically engineered biological substances. The proposed OPP guidelines on data requirements for biorational pesticides (including both biochemical and microbial pest control agents) could be a starting point for developing appropriate guidance. The OPP guidelines have received preliminary endorsements from outside groups that have examined them, but they would need to be adjusted to cover the types of substances and uses likely under TSCA. This would be an appropriate subject for further investigation.

c. Expertise of OTS staff

It appears that in many areas OTS would be lacking necessary staff expertise. Based on the experience of FDA in reviewing drugs produced through the use of rDNA, OTS would need microbiologists, biochemists and public health specialists with current work experience in the field to do the necessary health reviews. Also, the chemical engineering staff would need familiarity with containment practices for pathological organisms and the associated worker protection techniques, and the exposure evaluation and fate staffs would need to be able to estimate the likely endpoints of these substances and any degradation products once released into the environment. There would be a need for broad-scale ecologists who could look at potential long-term, indirect effects, such as the effects of microbial competition on the ecosystem and the food chain. OTS does not have staff of this type.

It could be possible to draw on OPP staff to assist in PMN reviews if the number of substances involved were not great. There are precedents for cooperation of this sort on an informal basis.

d. Risk assessment methodologies

The key problem for OTS is to develop reasonable risk assessment methodologies for genetically engineered substances and microorganisms. The unknowns are formidable, since it is not possible to know all the potential effects to look for. Risks may include (but are not limited to): infection, transfer of genetic material, and release of large volumes of biological substances into the environment without knowing what their direct or indirect effects would be on flora or fauna. It may be extremely difficult to evaluate the long-term and indirect effects of such substances. Risk assessment methodologies are therefore important to develop to prepare to deal with genetically engineered substances. High standards of proof may be the best safeguard.

7. Conclusions

Biotechnology is an area with great uncertainty in many respects. Industry is striking out in new directions to which it must become accustomed. Many government agencies have authority in this area. Public scrutiny is also growing. OTS is preparing to assume its regulatory responsibilities in this field but extensive analysis is still required.

There are many issues for OTS to consider in developing a policy on biotechnology. First and most important is the issue of whether microorganisms, (one of the three types of biological substances defined earlier) should be considered chemical substances under TSCA. This is the issue on which TSCA jurisdiction hinges. The preliminary work done for this paper strongly suggests that genetically engineered microorganisms and products made from them are subject to OTS regulation where they are used for TSCA purposes. However, the details of OTS jurisdiction, such as the appropriate statutory authorities to use, must be carefully investigated through internal analysis and coordination with other federal authorities.

A secondary set of issues, also very complex, is how EPA should prepare for the evaluation of these substances and what should be the nature and level of effort of OTS and Agency actions. The possibility of regulating genetically altered substances, perhaps even living organisms, poses significant and very complex issues with which OTS has never grappled. This includes issues like the nature and extent of possible risks; the time frame in which substances which TSCA might regulate will come to market; how to coordinate TSCA action with other federal authorities; and how to mobilize Agency resources to deal with these issues.

APPENDIX C

March 14, 1983

MEMORANDUM

SUBJECT: Status of Recombinant DNA and New Life Forms
Under TSCA

FROM: Stanley H. Abramson
Associate General Counsel
Pesticides and Toxic Substances Division (LE-132P)

TO: John A. Todhunter, Ph.D.
Assistant Administrator
for Pesticides and Toxic Substances (TS-788)

We have been asked informally by your staff to discuss whether TSCA grants authority to regulate recombinant DNA and new life forms. We think that the arguments in favor of regulatory authority are stronger than those against.

TSCA generally grants authority to regulate chemical substances.1/ Any DNA molecule, however created, is an organic substance of a particular molecular identity and is a combination of organic substances of particular molecular identities occurring in whole or in part as a result of a chemical reaction or occurring in nature.

1/ "Chemical substance" is defined in section 3(2) of TSCA as

> any organic or inorganic substance of a particular molecular identity, including--(i) any combination of such substances occurring in whole or in part as a result of a chemical reaction or occurring in nature, and (ii) any element or uncombined radical.

Similarly, a life form, whether a virus, a bacterium, or a more complex organism, is clearly comprised of "chemical substances" as defined in TSCA. Moreover, life forms are themselves "chemical substances" under TSCA because they are combinations of organic substances of particular molecular identities that occur in nature or that occur in whole or in part as a result of a chemical reaction.[2]

In addition to the statutory argument, one can also construct a strong policy argument for invoking TSCA authority. Congress intended TSCA to be a "gap-filler." No other federal regulatory scheme currently regulates life forms.[3] Thus, invoking TSCA jurisdiction over life forms would be consistent with the gap-filling function.

A decision not to invoke TSCA authority would also be supportable, although it would require arguing against the plain statutory language.

The argument would go as follows: Although DNA and life forms appear to fit the TSCA definition of chemical substances, there is no indication in either the statute or legislative history that Congress contemplated TSCA regulation of life forms.4/ The legislative history references numerous examples of problems which the statute was intended to address; life forms are not among them. Indeed, at the time Congress considered and passed TSCA, recombinant DNA research was in its infancy and seemed to have applications only in the pharmaceutical area. Furthermore, although the TSCA definition of chemical substances appears to include life forms, commonly accepted scientific definitions both at the time of TSCA's enactment and today do not. Finally, the provisions of TSCA do not lend themselves easily to regulating life forms. For example, one does not "manufacture" a life form in the same sense that one manufactures inanimate substances. Thus, one could argue that TSCA was not intended to regulate life forms.5/

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[2] This conclusion is further supported because life forms are not mixtures under TSCA. A mixture is a combination of chemical substances not occuring in nature and cannot be in whole or in part the result of a chemical reaction, except when the combination consists entirely of chemical substances on the Inventory and the ~~combination~~ could have been "manufactured for commercial purposes" without a chemical reaction. Thus any naturally occuring life form cannot be a mixture. In addition, all the chemical substances in any life form are not likely to be on the Inventory, and it certainly is very unlikely that one could produce a life form by combining the component chemical substances without using chemical reactions. Thus even an artificially produced life form cannot be a mixture.

[3] FIFRA would cover life forms used as pesticides; FDA would have authority over life forms used as, or in the production of, foods, drugs, or cosmetics. But no other statute or agency would have comprehensive authority.

For the reasons stated herein, we believe that the arguments in favor of regulatory authority are stronger than those against. If you decide that DNA and life forms should be subject to TSCA jurisdiction, there are a number of subsidiary issues (e.g. the line between research and development and other commercial activities and when a new life form is a new chemical substance) that I believe would best be addressed informally at the staff level at that time. We would be pleased to discuss the issues raised in the memorandum further should you so desire.

4/ It is difficult to think of any way that a DNA molecule would not be a "chemical substance" under the TSCA definition. However, a DNA molecule per se does not have any use aside from the life form of which it is a part. If the life form were excluded from TSCA jurisdiction, it might make sense to exclude its component DNA also. Thus, if the Agency were to conclude that a life form is not a chemical substance or mixture under TSCA, we could craft an argument that chemical substances which have a use only as part of the life form are not subject to TSCA jurisdiction.

5/ In the past the Agency has taken the position that DNA and life forms are chemical substances under TSCA, in documents such as the Inventory reporting instructions and in testimony during Congressional hearings on recombinant DNA research. While the Agency is not irrevocably bound to its earlier interpretations, any change should be explained and justified to avoid legal challenge.

LE-132P:JCNelson:jcn:3/14/83:x7213:WSMW517B

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 26 1983

OFFICE OF
GENERAL COUNSEL

MEMORANDUM

SUBJECT: Applicability of FIFRA or TSCA to Microbiological Agents Used to Control Ice Nucleation

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Office of the Assistant Admin-
for Pesticides and Toxic Subst

FROM: Stanley H. Abramson
Associate General Counsel
Pesticides and Toxic Substances Division (LE-132P)

TO: Don R. Clay
Acting Assistant Administrator for
Pesticides and Toxic Substances (TS-788)

This is in response to your request for a legal opinion on the applicability of either FIFRA or TSCA to two types of microbiological agents used to control ice nucleation. As detailed below, both substances fall within the FIFRA definition of pesticide, and hence, are subject to the requirements of FIFRA. Because they fall within the jurisdiction of FIFRA, it is not necessary to regulate these agents under TSCA. The Office of Pesticide Programs is currently developing guidelines for the regulation of biorational pesticides which appear to be applicable to these substances. 1/

A. BACKGROUND

The Agency has received several inquiries regarding EPA jurisdiction over two microbiological agents used to protect crops from frost damage. These agents may be used singularly or in combination to accomplish the same purpose. Because of the similarity of the issues involved and the possibility of the agents being combined in the same product, a single opinion was requested regarding both agents.

1/ On October 6, 1983, an ad hoc group of OPP and OTS personnel informally met to discuss their mutual concerns about genetically engineered substances and possible approaches to Agency regulation of them. For your information, I have attached a list of the participants as Appendix A, and note that the discussion was very helpful in the preparation of this memorandum.

Both agents act by manipulating the ice nucleation process on plants which is responsible for the formation of frost. The frost formation is mediated through ice nucleation-active (INA^+) bacteria which occur naturally on plants. Elimination or reduction of the INA^+ bacteria population allows plants to withstand lower temperatures without suffering frost damage. Accordingly, the ability to control INA^+ bacteria would be economically advantageous in certain growing areas where frost is a problem.

The first agent consists of ice nucleation-inhibiting bacteriophages which are naturally occurring viruses that, when applied to the crop, will invade and kill the INA^+ bacteria present on the crop surfaces. According to the U.S. patent for a plant protection process using these viruses, "[t]he bacteriophages selectively attack the ice-nucleating bacteria." Use of these bacteriophages is described as an alternative to chemical bactericides.

The second agent consists of genetically engineered ice nucleation-negative deletion mutants (INA^-) of the active bacteria. The INA^- bacteria are applied to the crops and displace the INA^+ bacteria by occupying the same spaces and using the same nutrients, thus achieving frost protection by competitively inhibiting the INA^+ bacteria population. In a request to NIH for permission to test INA^- bacteria under field conditions, the applicants describe the bacteria as a "frost damage biocontrol agent" which can be used as an alternative to "various bactericides, including antibiotics."

B. APPLICABILITY OF FIFRA OR TSCA

The Federal Insecticide, Fungicide, and Rodenticide Act as amended (FIFRA) grants authority to the Agency to regulate the sale, distribution, and use of pesticides. Section 2(u) defines a pesticide as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest." "Pest" is defined in section 2(t) as "any form of . . . virus, bacteria, or other micro-organism (except viruses, bacteria, or other micro-organisms on or in living man or other living animals) which the Administrator declares to be a pest under section 25(c)(1)." In regulations promulgated pursuant to FIFRA §25(c)(1), microorganisms (including bacteria) and viruses which exist under circumstances that make them deleterious to man or the environment are designated as pests. 40 C.F.R. §162.14.

Taking these definitions together makes it clear that the primary criteria for determining whether a substance is a pesticide are action and intent, rather than origin and mechanism of action. 2/ Both FIFRA and its regulations specifically contemplate inclusion of microbiological agents within the definition of "pesticide." Neither limits microbiological pesticides to naturally occurring microorganisms. Therefore, the fact that a substance is composed of or contains either a naturally occurring or a genetically engineered microorganism as an active ingredient is not controlling in the determination of whether that substance is a pesticide.

Simply stated, the threshold decision of whether a substance is a pesticide should be based upon whether the substance exerts a pesticidal action and whether its intended use is for its pesticidal effect. If both parts of the threshold question can be answered in the affirmative, then the substance is a pesticide under FIFRA. Once the threshold question is affirmatively answered, the Agency has numerous options from which to choose in determining the extent to which the substance should be regulated. Specific characteristics of the substance, such as origin and mechanism of action, are two important factors in the selection of options.

Relative to the two substances under discussion, it is clear that both fall within the FIFRA definition of pesticide. There can be little question that the INA$^+$ bacteria are "deleterious to man or the environment" when the frost caused by them disrupts or destroys crops which are valuable to man or wildlife. By definition, therefore, INA$^+$ bacteria are pests. Moreover, because

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2/ While this is a general statement of the basic criteria for a pesticide under FIFRA, it should be understood that these basic criteria may be subject to further explanation and interpretation (see, e.g., 40 C.F.R. §172.3). However, any further interpretation must be consistent with the statutory language and philosophy.

In addition, the Agency may elect not to regulate certain substances which arguably fall within the definition of pesticide. In some instances, these exemptions have been formalized in specific regulations (see discussion at footnote 5 below; also see 40 C.F.R. §162.5 exempting certain biological pesticides under FIFRA §25(b)(1)); in others, the exemptions have been more in the nature of informal policy decisions (e.g., the Agency does not regulate the ivy which is planted to keep down weeds in a lawn).

there is already a §24(c) registration for a substance which controls frost by inhibiting bacterial ice nucleation agents, there is at least a strong presumption that the Agency considers INA^+ bacteria a pest. 3/

Once it is established that INA^+ bacteria are pests under FIFRA, it is a logical progression to conclude that a substance which controls the pest, and which is intended to be used for that purpose, is a pesticide. Such is the case with these ice nucleation inhibitors. In both instances, the substances act to control INA^+ bacteria -- the bacteriophages through destruction and the INA^- bacteria through mitigation. From documents produced by proponents of these substances, it is clear that both are intended to be used to control INA^+ bacteria. (In fact, this use appears to be the only reason for producing these substances.) Accordingly, because both criteria for a FIFRA pesticide - action and intent - are satisfied, the INA^+ bacteriophages and the INA^- bacteria are pesticides and fall within the jurisdiction of FIFRA.

To the extent that these specific substances are pesticides under FIFRA, they are not "chemical substances" subject to regulation under the Toxic Substances Control Act (TSCA). See TSCA §3(2)(B)(ii). However, if these same substances were to be used for other, non-pesticidal, purposes, they may under certain circumstances fall within the jurisdiction of TSCA.

C. REGULATION UNDER FIFRA

The Office of Pesticide Programs is currently developing guidelines to govern the regulation of "biorational" pesticides. These guidelines appear to be applicable to both the naturally occurring and the genetically engineered ice nucleation inhibitors here discussed. Biorational pesticides include both biochemical and microbial pest control agents. In addition, the proposed regulations dealing with data requirements for the registration of biorational pesticides not only address bacteria and viruses, but also specifically address requirements for genetically-engineered microbial pest control agents. 47 Fed. Reg. 53192, 53202 (November 24, 1982); see also 44 Fed. Reg. 28093 (May 14, 1979).

3/ The state registration for Kocide 101 (EPA Reg. 8901-1), which has a chemical rather than biological active ingredient, was granted in 1982 apparently without objection by the Agency. Pursuant to FIFRA §24(c), if the Agency does not raise timely objections to a state registration, "[s]uch registration shall be deemed registration under section 3 for all purposes of this Act."

Generally, the extent to which the Agency elects to regulate substances designated as pesticides under FIFRA is largely dependent upon the potential risks related to their use. 4/ In both instances, proponents of these biological ice nucleation inhibitors claim that the substances will not have adverse effects on man or the environment. 5/ However, all of these safety claims must be fully assessed before the Agency makes a determination on whether and to what extent to regulate these specific pesticidal agents.

In summary, both the naturally occurring and the genetically engineered ice nucleation inhibitors referred to in your June 27, 1983 memorandum fall within the FIFRA definition of pesticide, and therefore fall within the jurisdiction of FIFRA. When used as pesticides, they are not "chemical substances" subject to regulation under TSCA. The Office of Pesticide Programs is currently developing guidelines to govern biorational pesticides which appear to be applicable to both of the substances here discussed.

4/ Of course, if it can be shown that use of these agents will cause no (or negligible) adverse effects, the Agency may choose to exempt them from all requirements of FIFRA under §25(b)(2). Section 25(b)(2) of FIFRA gives the Administrator discretion to "exempt from the requirements of this Act by regulation any pesticide which he determines . . . to be of a character which is unnecessary to be subject to this Act in order to carry out the purposes of this Act." Recently, the Agency [has used] §25(b)(2) to exempt certain pesticide products which contain, as the active ingredients, biological pheromone attractants on the basis of their low risk potential. 48 Fed. Reg. 38573 (August 24, 1983).

5/ The patent for the virus process asserts that "the bacteriophage inhibitors . . . are harmless to any living organism other than the specific ice-nucleating bacteria on which they are predators, . . . [that they] leave no residue that collects in the environment . . . , [and that they have no] known side effects to plants or animals." In petitioning NIH for approval for field trials of INA⁻ bacteria, the applicants claimed that their INA bacteria are not generally phytotoxic, are not very persistent in soil, and are not very likely to cause adverse effects in humans. (These arguments were apparently persuasive for the NIH review panel, because approval for the field trials was granted.)

THE SECRETARY OF HEALTH, EDUCATION, AND WELFARE
WASHINGTON, D.C. 20201

FEB 26 1979

The Honorable Bob Bergland
Secretary of Agriculture
Washington, D.C. 20250

Dear Bob:

As you know, I announced in December several steps we are taking with respect to the future course of recombinant DNA research in this country. Our objective is to permit the maximum freedom for scientific inquiry consistent with protection of the public health and the environment.

The recombinant DNA research documents published in the Federal Register on Friday, December 22 did the following: (1) approved revised Guidelines prepared by the National Institutes of Health governing safety requirements for the conduct of recombinant DNA research by institutions receiving NIH funding for such research; (2) directed NIH to increase its studies on the extent of risk associated with such research; (3) broadened public representation on the HEW advisory committee which will assist NIH in administering the revised Guidelines; and (4) increased public access to information about recombinant DNA research and public participation in administration of the Guidelines in local areas.

If the health, environmental, and ethical concerns raised by recombinant DNA research are to be accommodated effectively, these actions by the Government, and by those who receive Government support for recombinant DNA research, will not be enough. It is also necessary that research conducted by the private sector comply with the same Guidelines. To this end, I have directed the Food and Drug Administration to use its regulatory authority to require that all recombinant DNA research submitted to FDA to satisfy its regulatory requirements also comply with the NIH Guidelines. I am enclosing FDA's Notice of Intent to Propose Regulations for this purpose.

However, there may be significant recombinant DNA research conducted in the private sector which would not result in submissions to FDA. For this reason, I have sent a letter to Doug Costle, Administrator of the Environmental Protection Agency, asking him to review EPA's regulatory authority and take all action possible to require that recombinant DNA research conducted by the private sector comply with the NIH Guidelines. It has also been suggested that Agriculture might have authority to regulate privately supported recombinant DNA research in the agricultural area. If this is so, I would appreciate your considering how this authority might be used to assure compliance with the Guidelines.

If FDA, EPA, and USDA regulate recombinant DNA research in the private sector, virtually all recombinant DNA research in this nation will comply with the revised NIH Guidelines, assuring protection of the public health and the environment.

Sincerely,

Joseph A. Califano, Jr.

Enclosure

DEPARTMENT OF AGRICULTURE
OFFICE OF THE SECRETARY
WASHINGTON, D. C. 20250

HEW-OS
JUL 2 9 33 AM '79

June 27, 1979

Honorable Joseph A. Califano, Jr.
Secretary of Health, Education, and Welfare
Washington, D. C. 20201

Dear Joe:

I have reviewed your letter of February 26 regarding the regulation of recombinant DNA research in the private sector. As you know, Agriculture supports the regulation of recombinant DNA research under the revised guidelines. The Joint Council for Food and Agricultural Sciences, through its Recombinant DNA Committee, is developing procedures for assuring that all research in agriculture funded by USDA and the States is in compliance with the guidelines. The authority of the USDA does not directly extend to the private sector for regulation of recombinant DNA research.

USDA has authority under various laws, commonly referred to as the Plant Quarantine and related laws, to prevent the introduction into the United States of plant pests and pathogens and to prevent the interstate movement of various plant pests and pathogens not widely prevalent within and throughout the United States. Therefore, to the extent any products of recombinant DNA research or organisms containing recombinant DNA molecules could be classified as plant pests or plant pathogens, they would be subject to regulation in accordance with the Plant Quarantine and related laws.

Additionally, USDA has authority under various laws, commonly referred to as the Animal Quarantine and related laws, to regulate and take such measures as are proper to prevent the introduction into the United States or dissemination interstate of the contagion of any contagious, infectious or communicable disease of livestock or poultry. Further, USDA has authority to regulate the movement of animals affected with or exposed to any communicable disease of livestock or poultry in interstate or foreign commerce. Therefore, to the extent any products of recombinant DNA research or organisms containing recombinant DNA molecules could be classified as a contagious or communicable disease of livestock or poultry, they would be subject to regulation in accordance with provisions of the Animal Quarantine and related laws.

Also under the Virus-Serum-Toxin Act (21 U.S.C. 151-158), the Department licenses manufacturers of veterinary biologics which operate in interstate commerce and to the extent that recombinant DNA research occurs in these facilities, it could be monitored by the Department. However, those facilities which operate in intrastate commerce are outside the jurisdiction of the Department.

There are, of course, other authorities which might have an impact in this area, such as the Federal Meat and Poultry Inspection Acts (21 U.S.C. 601, *et. seq.* and 21 U.S.C. 451, *et. seq.*). These laws are mainly concerned with providing wholesome and safe meat and poultry products to the public. Any meat or poultry products which are "adulterated", within the meaning of that term, would come under the prohibitions of said Acts. If such "adulteration" were related to the administration of products resulting from recombinant DNA research or to exposure to organisms containing recombinant DNA, then the provisions of these Acts could have significance with respect to the area of recombinant DNA research.

Sincerely,

Bob Bergland
Secretary

THE SECRETARY OF HEALTH, EDUCATION, AND WELFARE
WASHINGTON, D.C. 20201

DEC 15 1978

The Honorable Douglas M. Costle
Administrator
Environmental Protection Agency
Washington, D.C. 20460

Dear Doug:

I am announcing today several steps we are taking with respect to the future course of recombinant DNA research in this country. Our objective is to permit the maximum freedom for scientific inquiry consistent with protection of public health and the environment, and with the profound ethical concerns raised by recombinant DNA research.

As noted in the public statement which is enclosed, I am today, (1) approving revised guidelines prepared by the National Institutes of Health governing safety requirements for the conduct of recombinant DNA research by institutions receiving NIH funding for such research; (2) directing NIH to increase its studies on the extent of risk associated with such research; (3) broadening substantially public representation on the HEW advisory committee which will assist NIH in administering the revised guidelines; and (4) increasing significantly public access to information about recombinant DNA research and public participation in administration of the guidelines in local areas.

If the health, environmental and ethical concerns raised by recombinant DNA research are to be accommodated effectively, these actions by government, and by those who receive government support for recombinant DNA research, will not be enough. It is also necessary that research conducted by the private sector comply with the NIH guidelines.

To this end, I have directed the Food and Drug Administration to use its regulatory authority to require that all recombinant DNA research submitted to FDA to satisfy its regulatory requirements comply with the NIH guidelines. I have enclosed FDA's Notice of Intent to Propose Regulations for this purpose.

However, there may be significant recombinant DNA research conducted in the private sector which would not be submitted to the FDA. For this reason, I hope you will review the Environmental Protection Agency regulatory authority and take all action EPA can to require that recombinant DNA research conducted by the private sector complies with the NIH guidelines.

If both FDA and EPA regulate recombinant DNA research, virtually all recombinant DNA research in this nation will comply with the revised NIH guidelines which assure protection of the public health and the environment.

I appreciate your willingness to work with us in this task.

Sincerely,

Joe

Joseph A. Califano, Jr.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB 25, 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Dr. William Gartland, Jr.
Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20205

Dear Dr. Gartland:

We at the Environmental Protection Agency (EPA) would like to respond to your request for comment (FR, December 7, 1982) on the progress made with determining the risks of recombinant DNA research. It is our opinion that great strides have been made to address the initial concerns about potential hazards from work in biotechnology, but we think there are a number of unknowns still remaining that require further inquiry on the part of the research community at large.

The recombinant DNA activities at NIH and the guidance provided by the Recombinant Advisory Committee focused at the logical beginning--research in academic laboratories and the consequences of direct exposures to genetically engineered organisms. With time, research activities expanded into industrial settings, and we are now beginning to see the fruits of their labor in the form of commercial products. Not only will we eventually have more chemical products synthesized by life forms, but also we will have the development of new agents that will be disseminated to the environment, either by willful release or by inadvertant escape.

It seems to us that there needs to be an expansion in the scope of the research dealing with potential public health and environmental risks of biotechnology:

1. More attention needs to be given to the consequences of inadvertent release of genomes from laboratories or facilities.

2. Although it was relevant to focus primarily upon the enfeebled E. coli in the past, more and more work is underway or being planned on spore-forming and free-living microorganisms. Some genera now being investigated include species that are known human, animal and plant pathogens. Therefore, studies should investigate the chance of survival of engineered organisms and the likelihood of their genetic exchange with other life forms.

3. An understanding is needed of the possible infectivity, pathogenicity and toxicity of microbes in non-target species.

4. Competition and possible perturbations of ecosystems by genetically engineered organisms of ecosystems should also be examined.

5. Methods to monitor for the presence of microbes in various environmental media and organisms should be developed,

6. Biological techniques to limit the spread of microbes in the environment need investigation.

We recognize that some of these remaining research questions listed above fall within the realm of EPA, but a number of them certainly overlap the missions of the NIH. Still others are relevant to the expanded Department of Health and Human Services and the Departments of Agriculture, Energy and Interior. Because of the interrelationships among agencies, we think there would be merit in broadening the scope of the risk assessment work and to make it into a more collaborative venture.

The EPA is charged by Congress to protect public health and our environment through a number of different statutes. We are concerned with contamination of water, drinking water and air, with solid waste and waste sites, and with pesticides and toxic substances. There is an impression that some of the commercial activities in the recombinant DNA field are being developed without the benefit of criteria for judging general population and environmental safety of the cellular products and especially the life forms themselves. We at EPA are charged with developing responsible public policy. Currently, it would seem prudent to investigate "potential" hazards associated with recombinant

DNA developments for which we lack sufficient information to substantiate or refute our concerns. Therefore, we hope that further risk assessment research of an expanded nature will be undertaken; we are eager to work with you and other agencies in developing a strategy; and we support an annual review of the progress made in the area of risk assessment work.

Sincerely yours,

Courtney Riordan, Ph.D.
Acting Assistant Administrator
for Research and Development

John A. Todhunter, Ph.D.
Assistant Administrator
for Pesticides
and Toxic Substances

cc:
TS-788 (2)
RD-672

Chapter 40—Protection of Environment

Executive Order 11987—Exotic organisms

SOURCE: The provisions of Executive Order 11987 of May 24, 1977, appear at 42 FR 26949, 3 CFR, 1977 Comp., p. 116, unless otherwise noted.

By virtue of the authority vested in me by the Constitution and statutes of the United States of America, and as President of the United States of America, in furtherance of the purposes and policies of the Lacey Act (18 U.S.C. 42) and the National Environmental Policy Act of 1969, as amended (42 U.S.C. 4321 *et seq.*), it is hereby ordered as follows:

SECTION 1. As used in this Order:

(a) "United States" means all of the several States, the District of Columbia, the Commonwealth of Puerto Rico, American Samoa, the Virgin Islands, Guam, and the Trust Territory of the Pacific Islands.

(b) "Introduction" means the release, escape, or establishment of an exotic species into a natural ecosystem.

(c) "Exotic species" means all species of plants and animals not naturally occurring, either presently or historically, in any ecosystem of the United States.

(d) "Native species" means all species of plants and animals naturally occurring, either presently or historically, in any ecosystem of the United States.

SEC. 2. (a) Executive agencies shall, to the extent permitted by law, restrict the introduction of exotic species into the natural ecosystems on lands and waters which they own, lease, or hold for purposes of administration; and, shall encourage the States, local governments, and private citizens to prevent the introduction of exotic species into natural ecosystems of the United States.

(b) Executive agencies, to the extent they have been authorized by statute to restrict the importation of exotic species, shall restrict the introduction of exotic species into any natural ecosystem of the United States.

(c) Executive agencies shall, to the extent permitted by law, restrict the use of Federal funds, programs, or authorities used to export native species for the purpose of introducing such species into ecosystems outside the United States where they do not naturally occur.

(d) This Order does not apply to the introduction of any exotic species, or the export of any native species, if the Secretary of Agriculture or the Secretary of the Interior finds that such introduction or exportation will not have an adverse effect on natural ecosystems.

SEC. 3. The Secretary of the Interior, in consultation with the Secretary of Agriculture and the heads of other appropriate agencies, shall develop and implement, by rule or regulation, a system to standardize and simplify the requirements, procedures and other activities appropriate for implementing the provisions of this Order. The Secretary of the Interior shall ensure that such rules or regulations are in accord with the performance by other agencies of those functions vested by law, including this Order, in such agencies.